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CELL REACTIONS[1]

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IT was my first thought to speak on cell behavior, but it becomes immediately evident that behavior results only from reactions in orderly sequence in time and space. The reactions themselves have long been within the technical range of the experimental biologist, and there has even been the beginning of an approach to the study of the orderly sequence that results in patterns both in organization and in behavior. The discovery of organizers gives a welcome glimpse into a world of mystery, just as the earlier studies on hormones threw some light on the machinery of the apparently incomprehensible harmony in bodily relations and behavior of the parts of the individual.

That single cells take an active but apparently regulated and controlled part in carrying on the bodily differentiation and activities of the individual; that such cells stand in somewhat the same relation to the life of the individual as the differentiated members to a community such as that maintained by certain social insects; that their activities create survival values not for themselves but for the individual of which they form a part—these propositions constitute my subject to-night. A few examples, familiar enough no doubt to most of you, will illustrate my meaning.

For my purpose, cell reactions may be considered to be intra-cellular, intra-organismal or extra-organismal. Of

[1] Address of retiring president, American Society of Naturalists, Columbus, Ohio, December 30, 1939.

reaction following an intra-cellular stimulus I will cite one example.

In the red alga *Agardhiella* the egg cell is very small and poor in cytoplasm and reserve food. After fertilization the fusion nucleus thus finds itself rich in the potentiality of reproduction, but poor in the materials necessary to make this reproduction effective. The sperms are non-motile and not particularly numerous, so that only a small fraction of the eggs are ever fertilized. There are present in the thallus other cells larger than the eggs and rich in protoplasm and in food materials corresponding to the yolk of the animal egg. These cells, known to the botanist as auxiliaries, lie at some distance, perhaps several millimeters, from the egg.

In this situation the egg sends out a filament, the oöblastema. The fusion nucleus moves to the tip of this filament and there divides once by a modified mitosis. The oöblastema creeps forward in the thallus without demonstrable chemotropic direction, touching cells here and there as it pushes its way through the soft gelatinous chinks between the body cells until it touches, as if by accident, an auxiliary cell. The tip then bends toward the auxiliary and fuses with it, and the two fusion or diploid nuclei pass into the rich cytoplasm found there. The haploid nuclei already present disintegrate, leaving the diploid nuclei from the egg plus the cytoplasm of the auxiliary to replace the original egg in producing the new individuals.

The stimulus of the entry of the diploid nuclei has a remarkable effect on the activity of the auxiliary. It proceeds at once to divide repeatedly and forms finally a spherical mass of spores, numbering several hundred and each of large size and provided with an abundance of stored food. This activity of the auxiliary is associated with a hardiness unusual in such delicate cells as characterize the red algae. In one specimen, laid open and placed in sea water on the slide just as the oöblastema filament was making contact with the auxiliary, it was noted that the vegetative cells of the body, including those

auxiliaries which had not received diploid nuclei, were all dead, their chloroplasts laked like blood corpuscles, within 30 minutes. The auxiliary, however, remained healthy and continued to divide under most trying conditions for seven hours.

The stimulus here is the presence of the fusion nucleus, the problem is to manipulate it in such a way as to insure effective reproduction. Transfer of nuclei for considerable distances in plants is seen in pollen tubes, but the red algae are unique in moving the nucleus from the egg itself to an entirely different cell some distance away. This manipulation of the egg nucleus plus cooperation of the auxiliary greatly increases the production of new individuals following a successful act of fertilization and thus compensates for the frequent failure of fertilization to take place. The number of egg cells is much greater than that of auxiliaries so that the wastage in the frequent failure of fertilization is minimized.

Of intra-organismal stimuli I may mention the unexpected behavior of cambium cells as shown by Bailey.[2]

In the white pine the dimensions of these cells have been given as 4,000 by 42 by 12 micra. Division planes along the short axis would be confidently expected by the cytologist. Actually division is in the plane least to be considered possible, and the long thin sheet-like cell is split into two still thinner sheets of the same length and breadth. In this unusual behavior, which in its defiance of physical laws is almost like making water run up hill, the cambium cell, of course, is reacting to some stimulus the nature of which is entirely obscure. The result, however, is a happy one, producing the very long cells needed in the conducting system of the stem.

The formation of the spicule in certain fresh-water sponges[3] is another example of cooperative action by which cells attain what may fairly be called an objective of survival value to the sponge. A single cell, the spicule-forming cell or primary silicoblast, elongates and lays

[2] *Am. Jour. Bot.*, Vol. 7, 1920.

[3] Schröder, *Zeitsch. f. Morphologie u. Oekologie der Tiere*, Vol. 31, 1936.

down an axial filament in the cytoplasm, the cell later dying. This filament is coated with silicic acid. After the silica content of the spicule-forming cell is used up in this way, amebocytes with large vesicular nuclei move up. These cells contain large clear vacuoles which come to show under dark-field illumination the same glassy appearance as the silica of the spicule itself. The amebocytes are secondary silicoblasts. One moves to a spicule cell, joins it and then pinches off the vacuole containing silica gel, along with some cytoplasm. The silica vacuole is left on the young spicule and gradually spreads over it, increasing its thickness.

When the spicule reaches a certain length, a second type of amebocyte, without a vacuole and with a small granular nucleus, moves to the spicule and joins it. As many as eight may become attached to the spicule. They transport it to its place of functioning in the body of the sponge.

Here we have three different kinds of cells cooperating to form and transport the spicule. One lays it down, another brings up and delivers silica to it, the third transports it to its functional destination.

Even more remarkable is the response of certain cells of the flatworm Microstomum to the nematocysts of Hydra.[4]

There are four types of nematocysts in the species of Hydra studied. Two of these, the penetrants and streptolines, when discharged penetrate the body of the victim or enemy. Upon discharge, both thread and bulb are shot out from the Hydra's body. The other two discharge only their threads, leaving the bulbs still embedded in Hydra's ectoderm. In one of them, the stereoline, the end of the thread is adhesive and may become affixed to the surface of the victim. In the other, the volvent, the thread acts as a lasso, coiling around a hair or other minute projection of the victim's body. Obviously, the penetrants and streptolines are offensive-defensive, while the

[4] Kepner, Gregory and Porter, in *Zoöl. Anzeiger*, Vol. 121, 1938.

stereolines and volvents are functional in capturing and holding prey until it is dragged into the mouth.

Microstomum kept for 50 generations without access to Hydra will eagerly ingest tissue of the polyp when it is presented. The fate of the ingested nematocysts has been carefully followed. All four types are first taken in by the non-glandular cells of the enteric epithelium or endoderm, where they lie in large vacuoles. These pass through the basement membrane of the endoderm and come to lie in the intercellular spaces of the mesodermal parenchyma. Here the stereolines and volvents are digested, but the penetrants and streptolines are engulfed by attending parenchyma cells and transported by them outward to the epidermis. The attending cell is then encapsulated by a second parenchyma cell. It loses much of its chromaticity during these movements and shows an irregular, shrunken nucleus, while the capsule cell stains normally. When the transporting cell comes into contact with the epidermis of Microstomum, the latter becomes deeply pitted both internally and externally, but does not rupture. The nematocyst is oriented within the transporting cell in such a manner that it points outward just as it did in the Hydra from which it came. It is also functional just as in Hydra and serves as an offensive-defensive weapon. It should be noted that the stereolines and volvents, which could be of no use to Microstomum, are digested within the parenchyma.

In this case we see a thoroughgoing cooperation among the cells of Microstomum in the manipulation of nematocysts of Hydra. The endoderm cell takes them in and passes them into the mesodermal parenchyma. Here the cells distinguish between the useful and non-useful kinds, digesting the latter and transporting the former to the point of functioning and orienting them there. Then the cells of the epidermis respond by producing a pit through whose thin membrane the nematocyst discharges its stinging thread upon suitable stimulation.

Considering the various examples of the striking complexity of the beautifully ordered reactions of cells, all

leading to the development of survival values to the individuals, though the cells themselves may perish, one sees an analogy to the social insects. In a hive of bees, each individual has some task and does it with the end result that the swarm survives. It has been said that the "spirit of the hive" directs and controls the activities of the individual, heedless of individual welfare, but most careful of survival of the species. Certainly the behavior pattern of the swarm and of the cells is very similar. In the case of the cells, perhaps some organizer or group of organizers may be found to be responsible for the complex set of cooperative reactions. That some force is directing the physical and chemical factors involved in the successive stimulations of cells to do some particular thing at a certain moment seems an inescapable conclusion. It is just as likely mathematically that Microstomum without directive control can manipulate Hydra nematocysts successfully and safely as it would be for a sonnet to emerge from a random serial selection of a certain number of letters from a jumbled pile of alphabetical characters.

In the third category the stimuli are extra-organismal, and here we have the best opportunity for their analysis. The most striking examples are found in insect galls of plants where, in response to stimuli apparently chemical in nature, most elaborate and beautifully effective structures are produced by various plant tissues. The "spirit of the hive" idea breaks down in this case, since the response by no means leads to the survival of the plant. Directive control of the cellular response comes from the insect larva, whose secretions must furnish the orderly succession of stimuli that bring about reactions similar in every respect to the normal life process itself. Growth, differentiation and form all emerge as a result of the larval activity. Spatio-temporal relations are established and maintained in the plant organ involved of the same nature as those seen in normal differentiation. I know of no material, animal or plant, that offers so likely a field of

attack on the most fundamental of biological problems. If some young physiologist, provided with the armamentarium afforded by the latest advances in biochemistry and biophysics, should undertake the analysis of the control exercised by the insect larva on plant differentiation, he might make a nearer approach to the mystery of life than has hitherto been possible.

One need not suppose, however, that such an investigation, be it ever so successful, will settle the perennial argument between such wholehearted protagonists as those distinguished physiologists, J. S. Haldane, on the one side, and the late Jacques Loeb, on the other. We would probably agree that there is a residuum for which no physicochemical description has yet been found. The question of whether it can ever be found is a philosophical rather than a biological one. The biologist can well stand aside from such a metaphysical problem and should certainly keep an open mind and not accept either the crass oversimplifications of some mechanists or the retreat from reality of some vitalists.

In the last 300 years this residuum has steadily been whittled down. Whatever a biologist believes in his heart, he must assume in his research that there is in this residuum, this "alogical core of the universe," no citadel that may not be taken. As Needham[5] says, "the minimization of the alogical core of the universe, then, is the proper pursuit of science."

To assume, however, as a basis of research that there is no unknowable is one thing, but it is quite a different thing to deny in daily life the reality of "the substance of things hoped for, the evidence of things not seen." The scientist has developed his special technique by a deliberate abstraction. The scientific method, the most wonderful tool ever produced by the mind of man, is based on this abstraction, by which we tacitly agree to assume that everything is spatial and therefore within the possible reach of the senses. It is not necessary for me to say to

[5] "Order and Life," p. 13, Yale University Press, 1936.

this audience that by use of this method man's comprehension of and control over nature has constituted a veritable grand slam, the only one perhaps to which he can lay claim throughout the long course of his struggle with nature. In our own memory, among the many far-reaching discoveries and applications of science to revolutionize our lives, it is said that the life expectancy has been raised by 21 years. Nevertheless, I get the impression from talking with many young people that the worthwhileness of life has sensibly diminished.

One of these young people might say: "You have shown that man is a natural object, subject to the same physiological laws as other animal organisms and part of a comprehensible natural order. You have shown that the actions of such animal mechanisms are nothing but tropistic responses, or at best conditioned reflexes; that the way of a dog with a bone, or with his master, or with other dogs, is of the same order as the way of a magnet with steel, or the behavior of an acid in the presence of a base. You have shown how inexorable tropisms (and taxies) are. You have stressed the analogies of living things. The dog howls when he is kicked for exactly the same reason that a bell rings when the rope is pulled. Therefore we are not responsible for what we do, since the immediate cause of all our actions is the tug of circumstance. We are at the mercy of every wind that blows, winds over which we have no control and about which we know nothing. There is no such thing as responsibility in an individual ridden by fate in all he does. It is not my fault when things go wrong."

Ladies and gentlemen, the relief rolls are based on that philosophy—the bodily bankruptcy that we see around us is a mirror image of a spiritual bankruptcy that is merely the reverse of a scientific philosophy whose obverse is bright and shining with hope for a better world.

Science has swept the field, but in doing so has extended to the daily life of people its own peculiar abstraction. Moral values belong to the unseen and probably the unsee-

able. To ignore the existence of values by leaving them outside the field of science has justified itself by many exciting results in the laboratory that seem to promise an ever more extensive control over the forces of nature and a clearer and wider understanding of the world and the universe.

There is, however, another side to the picture when the non-scientific public, somewhat dazzled by the solid results of research that so easily become the "miracles of science," begins to apply to everyday living the formula that has been so fruitful in research. Is it not true that the political, social and economic mess in which the world now finds itself may be traced to the same great abstraction that has meant so much to scientific research? It is only a very short step from saying, "The only reality is what we can see," to regarding a treaty as a scrap of paper having no values beyond the visible ones. Adolph Hitler, when he made his Munich promises in 1938, was thinking along the same lines as Bethmann-Hollweg in 1914 when he made his famous remark about the treaty guaranteeing Belgium against aggression. Both merely transferred to the field of *Weltpolitik* the technique useful in the field of science. That such transfer is dangerous is shown by the most casual glance at events in Russia, Poland and Finland in the last few months.

The repudiation of obligations when such repudiation becomes convenient has become a veritable disease, epidemic among the nations of the world. Even the most upright of the democracies has shown the symptoms. Our own country once obligated itself to pay certain bonds in gold or its equivalent. It became inconvenient to do so. By the science of economics it was concluded that ponderables would be conserved by a repudiation of the pledge given. Many suspect, however, that imponderables of far greater value were sacrificed than were compensated for by the money saved.

Napoleon is said to have stated that God is on the side having the heaviest artillery, but one of the most realistic

of nineteenth century statesmen, Bismarck, is quoted as saying that wars are won by imponderables. Now imponderables are not found in laboratories, but would it not be a service to clear thinking if we admit their existence elsewhere?

There is no indication that the age-old controversy will ever be settled. Perhaps by its very nature there is no solution, but most people lean to one side or the other. The attitude of the scientist may well be that of trial and error. We are forced back on the pragmatic. The best results seem to be attained by ignoring values in the laboratory and by recognizing them in that part of the world lying outside the ivory towers.

I am grateful to the society for this opportunity to appear before you. I am reminded of Sam McKeithan. A party of gentlemen went to fish on the Waccamaw River down in North Carolina. They were sitting around the fire after supper and telling tall tales of deep-sea fishing and the whoppers they had caught in the Gulf Stream. The guide sitting in the corner listened quietly for a while, then shifted his quid and spat, and remarked, "That reminds me of Sam McKeithan. There was a big fish in Lake Waccamaw, and some of us thought we'd catch it. We got us a plow line and got the blacksmith to hammer us out a hook. We took a plowshare for a sinker and a gum log for a cork and half a hog for bait. Well, we hadn't been fishing ten minutes when we got a strike. We all pulled, and we drug up to the bank the biggest fish you ever saw. 'Laws a mercy, boys,' says I, 'ain't that a pair of boots sticking out of his mouth?' So we caught hold of the boots, and out came Sam McKeithan. He wiped the mud and slime off his face, and he says to us, 'Gentlemen, I'm much obleeged.' "

Well, I am very much "obleeged," and I wish you all a very happy New Year.

LOCAL AND GENERALIZED DEFENSE REACTIONS IN PLANTS AND ANIMALS

LOCAL REACTIONS IN PLANTS[1]

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As in many subjects, there are a number of different sides to the problem of local responses of plants to parasitic attack, and the problem can be viewed from many different angles. So far, however, the greater emphasis has been laid on this problem by the plant pathologists, whereas the field has been sadly neglected by the plant physiologists. This means that these local reactions have been considered mainly from the standpoint of the parasite, whether or not the latter was facilitated or hampered by the response of the host plant. Of course, this side of the problem is most important to the pathologist and the disease-control officer who have to consider in the first place the spread of the disease and must devise methods of checking its growth. The plant physiologist, on the other hand, is more likely to ask how the host happens to react in such a specific way to the parasite. In his case, the parasite is not the problem, but only a means of studying certain reactions of the host. Therefore, what the pathologist takes more or less for granted, namely, the host's response to the parasite—is the specific problem of the physiologist. The latter is not much concerned with the question of the extent to which the reactions of the host plant control the parasitic attack, whether they are beneficial, whether they are useful adaptations, etc.

The limitations which are imposed on me are mainly limitations due to my own specialization as a plant physiologist and a time limit which prevents exhaustive discussion of the problem. Therefore we will take up a few special topics which seem to me of most interest and which

[1] Read at a symposium of the American Society of Naturalists in joint session with the American Society of Zoologists, the Botanical Society of America, the Genetics Society of America and Section N, the American Association for the Advancement of Science, Columbus, December 30, 1939.

give a rough survey of local reactions to parasitic attack in plants from the standpoint of the host, taking its growth reactions as a starting-point.

When consulting a text-book of plant pathology it will be evident that reactions to parasites run the whole gamut from death to stimulated development, and there are very few reactions which our fancy could imagine, that are not realized.

Let us start with the simplest case, namely, death, in which the parasite before or after penetrating the tissues kills the host cells. Let us distinguish in this case between obligatory parasites requiring living host cells—such as rusts, and the more saprophytic parasites which live only on the contents of dead cells. The latter parasites are least specialized and the local reactions of their hosts are simplest. In general, they cause small or larger injuries in the form of necrotic spots in mature tissues, or complete collapse of immature tissues in growing organs. Most of these parasites are unable to penetrate the cell walls and grow only between the cells in intercellular spaces or by dissolving the pectins of the middle lamella. To collect the food inside the cells of the host their semi-permeability has to be destroyed, and this is achieved by killing the cells. In many cases these parasites excrete specific toxins which diffuse in the host tissue, killing all cells as soon as their concentration has reached a certain limit. As soon as death of the host cell has occurred, their sugars and other diffusible food material will leach out and become available to the parasite which is waiting outside the cell wall of these cells. This simple reaction also explains why so many saprophytes follow in the wake of these less specialized parasites, for, when once a parasite has killed the host cells, any saprophyte can live on the freed cell contents. If the host cells are in excellent health the toxins are less effective and the plant may not be attacked. From a physiologist's standpoint, these fungi are of little interest. It is the crudest form of parasitism. If we compare these relations with our own social structure we can com-

pare this case with highway robbery. Just stab the victim and, when his resistance has been nullified by death, take whatever you want from him.

There is a somewhat more interesting angle to this problem when the parasite does not progress too rapidly inside the host tissue. Then the host may be able to form a layer of wound cork around the attacked region. In this case we have to do with an interesting physiological reaction which is already understood to some extent. Haberlandt had shown that wounded cells liberate diffusible substances which stimulate cell division. These he called wound hormones. In conjunction with lepto-hormones coming from the phloem they are responsible for cell divisions and the formation of, for instance, the cork layer at the cut surface of a potato tuber. One of these substances, traumatic acid, has now been isolated and synthesized and, presumably, the isolation of others will follow in time. With this knowledge at hand we can now form the following picture of the cork layer formed around the infected areas. Let us consider a parasite which has entered the plant, killing cells and liberating all cell contents, including wound hormones. The food is taken up by the parasite and the diffusing traumatic acid will stimulate the surrounding cells to divide. If the rate of killing by the parasite exceeds the rate at which traumatic acid can induce a protective cork layer, the infection will spread, but if, on the other hand, the traumatin has acted before the diffusing toxins have killed the cells a protective layer will be formed around the infected area. A very typical result of such cork-layer formation is the shot-hole disease in cherry leaves. The killed tissue around the parasite (*Coccomyces prunophorae*) has been surrounded by a cork layer and has been severed along this layer leaving small, round holes in the leaf. Also, sulfur dioxide and other gas injuries become surrounded by such cork layers. In the latter case it is easy to see how the cork layer could form since no spread of toxins prevents the cork formation.

In this case a very apt comparison can be made with

cities of the Middle Ages. Before they were surrounded by a protective earth-and-stone layer and gates they were periodically ransacked. But if they were not completely destroyed, they could build protective layers and the infestation was checked.

In the case of obligatory parasites, such as rusts, the conditions are practically reversed. The parasite can only live at the expense of the living cells of the host. Its hyphae penetrate the cell walls, and then their protoplasm seems to mix with that of the host. The more susceptible the host, the fewer necrotic cells. But when the host is resistant, it means that the rust, when it penetrates cells, kills them, which necrosis may spread so that typical discolored areas around each infection appear. The rust can not live on dead cells and, therefore, dies if the host cells are dead. Here the local reaction of the plant, leading to resistance, is of the simplest type. The parasite is of the beggar type. It can exist only as long as the host is sympathetic, but it has to succumb when the attitude of the prospective donor is unfavorable. It is a true symbiosis.

There are a number of such obligatory parasites which so far have resisted all attempts to grow them in culture solutions. Apparently they receive from the host plant substances which are destroyed upon the death of the host itself. In general these parasites are the most extreme in their specialization so that they are able to attack only one, or few species, and within the species again only certain varieties. In many cases they also have a most remarkable succession of fruiting stages which alternate on very different hosts. This specialization is a remarkable physiological problem and might be due to and enable the analysis of protoplasmic specificity, but so far plant physiologists have not followed up this problem. Perhaps it is still too complex to solve with our present knowledge and methods.

Having discussed the reactions leading to death of the host cells, we now have to consider those cases in which

the parasite modifies the development of growth rate or form of the host.

Reactions to simple wounds inflicted by browsing cattle or other herbivorous animals are quite interesting, but hardly fit into this discussion. Very seldom, if ever, will the response be different from those following experiments in which parts of the plant have been removed by cutting. Also, reactions to man-inflicted injuries, such as tree surgery, lawn mowing[2] and pruning, would come under this heading.[3]

Of great interest are all changes in form or growth rate or development in general, which are induced by parasites. In many cases structures develop which under normal conditions are not present in the plant. We only have to think of corn smut, nematode galls on roots, insect galls, bark canker, crown gall, to realize what remarkable effects parasitism may have on the host. Naturally, one asks whether the parasite is able to bring about completely new potentialities, which are not present as such in the host. The answer to this question can be given only after a much closer analysis of the facts. To begin with let us take the case of crown gall, a parasitic outgrowth common on many plants, which has been analyzed in considerable detail.

After a bacterium, *Phytomonas tumefaciens*, has entered the plant, usually through some wound, a tumor-like enlargement develops at the place of infection. The first symptoms may appear five days after infection but mostly a longer period elapses. The growth of the tumor may continue for a very long time, until large swellings have developed. The rate of growth of the swelling depends upon the condition of the host plant; when the latter is in a resting period, little or no growth of the crown gall oc-

[2] It might be pointed out, however, that some of these reactions are distinctly advantageous. A meadow which is regularly pastured or mowed has a completely different vegetation than when left untouched. Only those plants which can easily regenerate buds from roots, or in which the lower axillary buds can grow out after cutting the tops off, or which form prostrate shoots will survive.

[3] This all comes under the general heading of "survival of the fittest."

curs. A rapidly growing gall will inhibit the development of other galls on the same plant. This all indicates that the gall which is produced is a cooperative undertaking in which the host is not passive. The host not only furnishes all cells and food materials necessary for growth of the gall, but also its condition is reflected in gall growth. The tissue which is formed is generally rather undifferentiated callus tissue, which especially on woody hosts later may become woody through differentiation of wound tracheids. Sometimes roots or shoots will develop on the surface of these galls, giving rise to so-called "hairy root." All these reactions may occur on plants after simple injury, without bacterial infection. The callus formed on poplar cuttings, *e.g.*, resembles crown gall.

While all these facts already indicate that the effects of the crown gall parasite are not due to highly specialized activities, the relative simplicity of the parasite-host relations in crown gall is clearly demonstrated by the fact that on many plants application of high concentrations of certain growth-promoting substances will induce swellings which closely resemble crown gall infections. Although it has been demonstrated also that *Phytomonas tumefaciens* can form growth-promoting substances *in vitro,* the available evidence rather points to the conclusion that the parasite does not induce gall formation on the host by simply producing growth-promoting substances. In the first place, the auxin concentration produced by the bacterium is so low that if applied by itself it would not cause any effect. In the second place, non-pathogenic bacteria may form the same amounts of auxin. And in the third place, there are many plants which do not respond to auxin application but which produce large crown galls with Phytomonas.

At first sight one is inclined to consider the considerable outgrowth due to Phytomonas as a useful adaptation, and the ability of the latter to induce such excessive growth of the host as purposeful and advantageous. But such a general consideration meets with difficulties: the shoots and

roots developing from the crown gall are apparently not infected. Therefore, we have to admit that at least some of the responses following bacterial infection are accidental and not useful, and are a natural sequence of the reactions started by the parasite. This means that many reactions induced by the parasite are accidental rather than purposeful. And when we consider the gall as a whole, doubt arises as to the usefulness of the large outgrowth. Certainly the bacteria represent only a very minor fraction of the total bulk of tissue formed, and even if no major swelling occurred, would have sufficient food to draw upon from the host.

Since every plant pathologist knows how difficult it is to sterilize completely many living plant tissues, even when outside infection of healthy organs is practically excluded, we come to the conclusion that in many apparently healthy plants bacteria live, which, although drawing upon the food supply of their host, do not induce abnormal growth. Therefore it seems not unlikely that the reaction induced by the crown gall parasite is only, let us say, an accident, not essential for the proper growth and development of Phytomonas, but based on a constellation of accidental factors, which induces growth of the host, just as application of indole acetic acid causes cell growth.

Other abnormal growth reactions are caused by fungi which induce witches'-brooms. Under the influence of the parasite the normal inhibition of lateral axillary buds by the apical bud is abolished, and most laterals grow out. The mechanism of the formation of witches'-brooms has not been determined as yet, but it seems likely that it can be explained completely in the light of our present knowledge of bud inhibition as an auxin phenomenon.

Some remarkable reactions are caused by those parasites, which make organs develop where otherwise there would have been none. A typical case is that of *Ustilago violacea,* which causes the formation of anthers in the female flowers of *Melandrium rubrum,* a strictly dioecious plant. In this case, the parasite only brings out a poten-

tiality of the host species, which otherwise in the particular individual (female plant) does not express itself.

We will conclude this survey with a description of some malformations, etc., caused by animals. These may be due either to decreased or increased growth. A very simple malformation is the crinkling of leaves produced by early infection with aphids. These animals penetrate with their mouth parts some of the sieve tubes. If they attack young plant parts, then all growth due to auxins—that is, cell elongation of stem, petiole and leaf veins—is decreased. Mesophyll growth continues at a more normal rate, and this causes a bulging of the intercostal areas of the leaves, giving rise to the formation of pockets in which the aphids are well protected. The effect of such aphid infection can be explained satisfactorily by assuming auxin destruction in the phloem. In other cases, excessive elongation of cells or organs occurs, such as erineum (which sometimes can be induced by application of indole acetic acid), which can be attributed to increased auxin content of the reacting tissues.

But the most complex structures are the galls formed by gall midges and gall wasps. These galls are structures of determinate growth, mostly of very uniform size, and have forms which differ very much from anything normally produced by the plant. They are built out of tissues of the normal plant, but these have been organized in a very different and definite pattern.

The stimulus for this abnormal organization almost invariably comes from the egg or young developing larva of the gall insect, and except in the case of the Pontania or Capreae gall, not from the egg-laying insect. In some cases the larvae even crawl to the place where the gall is going to develop, so that the deposition of the egg has nothing to do with the gall. This is also demonstrated by the fact that if the gall wasp fails to lay eggs in the incisions it has made, or if the eggs are removed soon after laying, no gall develops. This all means, then, that the galls are

local responses to the activity of the larvae of the gall insects.

The complexity of the structures induced by the gall insects is often astounding. The central part of the gall with the insect in it may become detached after it is full grown. Then the insect will be released from this box through opening of a pre-formed lid. Often very special structures develop enabling the full-grown insect to escape from its perfectly closed larval chamber. The inside of this larval chamber is often lined with cells very rich in proteins.

Only in the case of the Pontania gall on willow leaves, Beyerinck found that it would develop even if no egg was deposited. But in this case the insect, or tenthredonid, injects a small amount of material from a special gland into the incision made with its ovipositor, and this by itself is sufficient to make the gall develop. Here we have the first published concrete example of a substance causing differentiation and growth, and Beyerinck named it a "growth enzyme."

In general, we can say that the study of those local reactions which we call galls will yield the most interesting material for analysis of developmental processes. With the example of the insect before our eyes, we know that influencing the normal growth of a plant is possible and we should not rest until we have imitated the gall wasps in their effects on plants.[4]

It does not seem to lie within the range of my subject to discuss certain cases of immunity due to preformed anatomical or physiological characteristics of the host plant, such as disease-escaping immunity, pH of cell sap which excludes chemotropic attraction of parasite, extra heavy cuticle, thorniness of branches which prevents birds depos-

[4] In the previous discussion we have met parasites of very different types, such as robbers and beggars. To round out our comparison, I propose to nominate the gall parasites as gold-diggers. For they are able to induce the host to excesses far beyond anything they provide for their own offspring and also far beyond the needs of the parasite. And, as in human society, certain plants, such as oaks, are notorious as suckers.

iting seeds of parasitic Loranthaceae, etc. Those are all defense mechanisms, but are not local reactions. They are of importance for the plant pathologist, but lie outside the field of the physiologist.

In the preceding discussion we have seen that there is a very wide variety of local responses of plants to parasitic attack, ranging from death of the cell to greatly increased growth and even the formation of apparently new forms. But in all cases the new formations are definitely within the potential range of development of the host, and in some of the simpler cases these new forms have been induced by chemically known substances. What the parasite does is to bring out potentialities, rearrange tissues, and develop new growth patterns. Here lies a rich field for cooperation between pathologist and physiologist.

GENERALIZED DEFENSE REACTIONS IN PLANTS[1]

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INTRODUCTION

SOME plants are naturally immune or resistant to diseases that attack other closely related plants. This natural immunity may be due to either morphological or physiological conditions that prevent entrance and subsequent growth of the parasite in the plant body. Many parasites are dependent upon the presence of natural or artificial openings in order to cause infection. When such openings are absent, the host is immune. On the other hand, the parasite may readily gain entrance to host cells but find conditions there unsuited to its further development. For example, the resistance of red and yellow onions to the onion smudge organism, *Colletotrichum circinans* (Berk.) Vog., was shown (Walker, 1923; Walker, Link and Angell, 1929) to be due to the presence of protocatechuic acid in the outer scales of these onions.

Although natural immunity in plants has been recognized for a long time, it is only in recent years that good evidence for the existence of acquired immunity has been obtained. In a symposium on defense reactions in plants and animals, it is appropriate to review this evidence. The review will be concerned only with virus diseases, since there is some question whether acquired immunity from bacterial and fungous diseases has been demonstrated in plants.

THE MEANING OF ACQUIRED IMMUNITY

At the outset, it is well to state clearly what is meant by the term "acquired immunity." It is the specific *resis-*

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tance an individual organism acquires to a disease. The fact that the resistance is specific differentiates it from changes in susceptibility induced by nutrition or aging of the host and by such environmental factors as would be apt to alter resistance not only to the disease in question but also to various other unrelated diseases. Acquired immunity may be either active or passive: It is active if induced by infection with a parasitic agent, passive if caused by injection with immune serum or antibodies obtained from other individuals. Passive immunity has not been demonstrated to occur in plant diseases.

It is convenient to classify active immunity into three types, the chronic-disease type, the carrier type and the sterile type. In the chronic-disease type, neither the immunizing disease nor the causal agent disappears, but the individual is solidly immune from closely related diseases. In the carrier type, obvious manifestations of the disease disappear, but the causal agent persists. However, the individual may be protected from a second attack of the disease even when subjected to massive reinoculations with the infectious agent. In the sterile type, the immunity may be transitory or permanent and readily or with difficulty broken down by reinoculation; both obvious signs of disease and the inciting agent disappear or at least can not be detected by ordinary methods. The sterile type has not been demonstrated for plant-virus diseases. It must be recognized that there is no sharp line of division among the three types of acquired immunity. The extent to which the pathogenic agent disappears determines whether the immunity is of the sterile or carrier type, and the extent to which symptoms disappear determines whether it is of the carrier- or chronic-disease type.

For the purpose of the present discussion it will be more convenient to divide acquired immunity into the immunity which follows recovery from disease, and cross immunity, in which one strain of a virus protects a plant from infection by closely related strains. Immunity following recovery from disease is usually of the carrier type while cross immunity is more commonly of the chronic-disease type.

Immunity Following Recovery from Disease

There are at least six different virus diseases from which plants recover and acquire immunity. These are tobacco-ringspot (Price, 1932), tomato-ringspot[2] (Price, 1936), tobacco streak (Johnson, 1936, 1937), Bergerac-ringspot of tobacco (Smith, 1937), sugar-beet curly top in certain races of tomato and in tobacco (Lesley and Wallace, 1938; Wallace, 1939) and potato yellow dwarf (Black, 1937, 1938). Of the six diseases, tobacco-ringspot has been the most extensively studied.

The virus of tobacco-ringspot is readily transmitted by rubbing juice from a diseased plant over the leaves of susceptible plants. Tobacco (*Nicotiana tabacum* L.) plants inoculated in this manner develop a characteristic type of lesion on inoculated leaves in about three days and systemic lesions of a similar type in three or four more days. The lesion consists of concentric necrotic rings alternating with normal green tissue. In from twelve to thirty-five days after inoculation, diseased tobacco plants begin to recover. Recovery consists in the production of new symptom-free growth and eventual loss of old affected leaves. The first indication of recovery is the development of leaves with necrotic patterns resembling the outline of an oak leaf. The basal portions of such leaves are healthy in appearance, while the apical portions frequently show zonate necrotic lesions. As new leaves are produced, the lesions and oak-leaf patterns are restricted to progressively smaller areas at the leaf tips. Finally, such symptoms fail to appear altogether. Subsequently produced leaves resemble those of healthy plants but are slightly darker in color, somewhat thicker and of a more leathery texture. Recovered plants appear to grow somewhat less rapidly than healthy plants. Wingard (1928) transmitted tobacco-ringspot to plants of thirty-eight genera in seventeen different families and reported that recovery from the disease occurred in practically all the plants tested. Re-

[2] This disease was previously referred to as tobacco-ringspot No. 2. The writer here uses the name proposed by Holmes (1939).

covery from tobacco-ringspot occurs under a wide range of environmental conditions (Price, 1932) and is different from the masking of symptoms (Johnson, 1921; Goss and Peltier, 1925; Bennett, 1927) of other virus diseases caused by exposure to special environmental conditions or by aging of the host plants.

Plants that have recovered from ringspot are solidly immune from a second attack of the disease (Wingard, 1928; Henderson and Wingard, 1931; Price, 1932). Plants grown under favorable environmental conditions from cuttings of recovered tobacco plants have been repeatedly inoculated with ringspot virus without the production of symptoms; whereas plants grown from cuttings of healthy plants and treated in exactly the same manner have invariably come down with the disease (Price, 1932).

By definition, acquired immunity is specific. Acquired immunity from tobacco-ringspot meets this criterion. Tobacco plants that have recovered from ringspot are susceptible to infection with tobacco-mosaic, cucumber-mosaic, potato-veinbanding, potato-ringspot, tomato spotted-wilt, tobacco-etch, tomato-ringspot, tobacco-streak, and Bergerac-ringspot viruses and, no doubt, to all other tobacco viruses that are not closely related to tobacco-ringspot virus (Price, 1936; Johnson, 1937; Smith, 1937). They are immune from or highly resistant to infection by closely related viruses such as those causing Valleau's (1932) green and yellow ringspot diseases.

That acquired immunity from tobacco-ringspot is of the carrier type is readily demonstrated by testing for the presence of virus in recovered plants (Wingard, 1928; Henderson and Wingard, 1931; Price, 1932). Indeed, the presence of virus in such plants led Valleau (1935, 1935a) to conclude that tobacco does not actually recover from ringspot. He maintained that symptoms are produced only when the growing point of the plant has not been completely invaded by virus, that leaves developed subsequent to invasion are parasitized although patternless, and that inoculation of patternless leaves is without effect since they

are already parasitized to the limit. Actually, however, it has since been demonstrated that virus does not multiply to nearly the same extent in recovered plants as in recently infected plants grown under the same conditions (Price, 1936; Stanley, 1939). The recovered portions of affected plants contain, on the average, only from 10 to 20 per cent. as much virus as the diseased portions of the same plant. Even in the same leaf, a partly recovered one, the diseased portion was found to contain considerably more virus than the healthy appearing portion. Furthermore, the virus concentration of recovered leaves is not increased by inoculating them heavily with ringspot virus.

Acquired immunity in tomato-ringspot, Bergerac-ringspot and tobacco-streak diseases is also of the carrier type and in other respects is similar to that observed in tobacco-ringspot (Price, 1936; Johnson, 1936, 1937; Smith, 1937). In each of these diseases, recovery is characterized by the appearance of oak-leaf patterns followed by production of leaves that are symptomless or nearly so. Recovered plants carry virus and are refractory to reinfection.

Recovery of tobacco and tomato from the curly-top disease (Lesley and Wallace, 1938; Wallace, 1939) may take place either by growth of healthy-appearing shoots from axillary buds or by production of progressively less severely diseased leaves from the terminal. In either case, the new growth shows a non-sterile immunity. In tomato, the immunity is not of so high an order as in the ringspot and streak diseases, since infected plants do not always recover and since those that do recover occasionally show a relapse and may, in a few instances, develop severe symptoms when reinoculated (Lesley and Wallace, 1938).

Recovery of potato plants from yellow dwarf likewise occurs by growth from axillary buds; such new growth shows chronic symptoms that persist throughout life. However, plants that have recovered from yellow dwarf in this manner may be grown through at least 5 vegetative generations without again developing acute symptoms of the disease (Black, 1937).

THE CROSS-IMMUNITY REACTION

Cross immunity in plant virus diseases may be either of the carrier or chronic-disease type. The protecting virus may be so mild as to produce no visible symptoms whatever or so severe as to cause marked distortion and chlorosis. In either case the plant cells are solidly immune from other closely related viruses, although fully susceptible to non-related viruses.

Thung (1931) was the first to study cross immunity in plant virus diseases. He isolated a yellow-mosaic virus from ordinary tobacco-mosaic virus and found that plants infected with the ordinary mosaic virus developed no change in symptoms when inoculated with the yellow-mosaic virus. Similarly, plants infected with the yellow-mosaic virus developed no change in symptoms when inoculated with the ordinary mosaic virus. Moreover, only one virus could be isolated from the doubly inoculated plants, the one first introduced. These results led Thung to conclude that only one virus could multiply extensively in any given cell.

Thung's results have been confirmed and extended by numerous investigators working with many different plant viruses. Perhaps the most striking example of cross immunity in plant virus diseases is that shown by *Nicotiana sylvestris* Spegaz. and Comes plants infected with certain strains of tobacco-mosaic virus. Kunkel (1934) found that mature leaves of *N. sylvestris* plants infected with mottling-type strains of tobacco-mosaic virus were immune from the necrotic-type aucuba-mosaic strain. That this immunity is specific was shown by the fact that leaves mottled by cucumber-mosaic virus or systemically infected with tobacco-ringspot virus were not, on the other hand, immune from the necrotic-type tobacco-mosaic virus.

Evidence that the cross-immunity reaction is strictly cellular was obtained in the following manner. When mature leaves of *Nicotiana sylvestris* plants, or portions of such leaves, were rubbed with mottling-type strains of tobacco-mosaic virus they became immune from the ne-

crotic-type strain of the virus within a period of two days. Those portions of the leaves that were not inoculated with the protecting virus did not become immune. This and similar evidence indicates that only those cells actually invaded by virus develop an immunity.

The cross-immunity reaction has been of considerable value in demonstrating virus relationships. As a case in point, acquired immunity of *Zinnia elegans* Jacq. from cucumber-mosaic virus is of interest. Mottled leaves of zinnia infected by any one of a number of different strains of cucumber-mosaic virus are immune from a necrotic-type strain (Price, 1935). Zinnia leaves mottled by other viruses are not immune from the necrotic-type cucumber-mosaic virus. It has been found that the viruses of Southern celery mosaic and lily mosaic both protect zinnia plants from the necrotic-type cucumber-mosaic virus (Price, 1935a, 1937; Ainsworth, 1938). This result led to the conclusion that both viruses should be classified in the cucumber-mosaic virus group, a conclusion supported by other lines of evidence.

The Nature of the Defense Mechanism

It seems clear from the evidence just summarized that plants can and do acquire immunity from certain virus diseases. The important question to be answered is: What is the nature of the defense mechanism involved in this immunity? Unfortunately, this question has not yet been answered. Nevertheless, it may be appropriate to discuss some of the theories that have been advanced to explain the acquired immunity.

Up to this point, I have spoken of acquired immunity in plant virus diseases as a generalized reaction. The fact of the matter is, however, that it appears to be generalized only because the viruses themselves are generalized; viruses usually become systemic a few days after their introduction into a plant. As mentioned previously, the available evidence indicates that the immunity is confined to those cells actually invaded by virus. Whether or not the immunity extends to nearby cells is, of course, a moot

question and not easily determined. It seems clear, however, that it does not extend further than, perhaps, the length of a few cells from tissues actually occupied by virus. It thus appears that acquired immunity in plant virus diseases is cellular in nature.

In the case of tobacco-ringspot, in which immunity follows recovery of plants from the disease, it has been postulated (Price, 1936) that the ringspot virus is able to reach its maximum concentration in, and to exert its maximum effect upon, only those cells that are mature or nearly so at the time of invasion. According to this view, invasion of embryonic cells is not accompanied by maximum increase in virus nor by severe injury to the cells; the embryonic cells become adapted to the virus and maintain an equilibrium with it. Hence, the plant recovers as soon as the embryonic cells become infected. Assuming this hypothesis to be correct, it still does not reveal the nature of the mechanism involved.

A more fundamental hypothesis for the nature of the defense mechanism is based on the assumption that certain protoplasmic constituents, such as, perhaps, specific amino acids, are essential for virus reproduction (Kunkel, 1934; Köhler, 1934; Bawden, 1934; Salaman, 1936). This being the case, one virus in a plant may exhaust the supply of material essential for reproduction of that particular virus and thus render the invaded cell immune from closely related viruses that require the same material. The cell would still be susceptible to unrelated viruses, since these viruses would not require exactly the same materials as the first virus. This hypothesis may serve to explain the cross-immunity reaction, but it does not explain the immunity which follows recovery of plants from tobacco-ringspot disease. In this case, the virus content of recovered tissue is considerably lower than that of diseased tissue, and it is to be inferred that either the essential material has not been used up or its production has been sharply curtailed.

The suggestion has been made (Thung, 1936) that virus-

infected plants, like animals, develop immunizing substances which neutralize or tend to neutralize the virus in the plant. In fact, Wallace (1939a) recently reported an experiment which he believes furnished evidence of production of antibody-like substances in Turkish tobacco plants infected with curly-top virus. When the virus is transmitted from recovered plants to healthy plants by means of the insect vector a typical severe disease is produced. But if the virus is transmitted by grafting scions from recovered plants onto healthy plants only mild symptoms usually result. The inference is that the grafting process allows transmission of immunizing substances as well as the virus, whereas the insect transmits only the virus. However, there are other explanations for production of only mild symptoms following grafting. It is possible, for example, that plants recovered from curly top contain both mild and severe strains of virus and that the grafting process transmits both strains while the insect transmits only the severe strain. Until such possible explanations have been eliminated it seems unwise to conclude that curly-top-virus antibodies are produced in plants.

When tobacco plants that have recovered from the ringspot disease are grafted onto healthy plants, acute symptoms of the disease are produced in healthy plants. This indicates that antibodies, if they are indeed present in recovered plants, are not transmitted through the graft union (Price, 1932). Recently, experiments were made specifically to test for the presence of neutralizing antibodies in plants that had recovered from the ringspot disease. On the assumption that antibodies might be present in juices of such plants, several treatments were resorted to in an attempt to separate the virus from the antibodies. Juice from recovered plants and juice from diseased plants were subjected to the same treatment. These juices were then added in various proportions to juice from diseased plants; the resulting mixture was allowed to stand for an interval of time and was then tested for virus concentration by inoculation of 40 leaves of the

cowpea (*Vigna sinensis* Endl. var. Black). The data obtained from these experiments are summarized in Fig. 1.

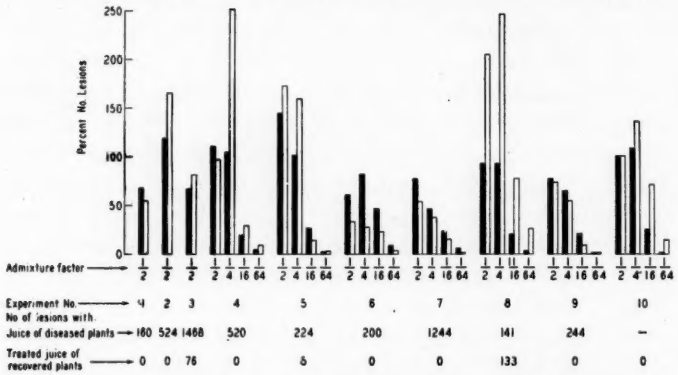

FIG. 1. Results of attempts to demonstrate neutralizing antibodies in plants that had recovered from tobacco-ringspot. Black columns represent relative numbers of lesions produced on sets of 40 cowpea leaves by diseased-plant juice diluted with various amounts of healthy-plant juice; white columns, by diseased-plant juice diluted with various amounts of recovered-plant juice that had been treated to remove virus. In each case the number of lesions is shown as percentage of the number produced by undiluted juice of diseased plants.

In experiments 1–6, juices of recovered and healthy plants were heated to 60 or 70° C. for 10 minutes before mixing them, in proportions up to 64 to 1, with juice of diseased plants; in experiments 7–9, the juices were filtered through a Berkefeld W filter candle before mixing; and in experiment 10, the virus-free supernatant from an ultracentrifuged sample of juice was used. The presence of antibodies in the treated juice of recovered plants would be expected to lower the infectivity of the virus to which it was added. Also, the reduction should be more pronounced as the proportion of recovered-plant juice was increased. Study of Fig. 1 shows that the addition of juice of recovered plants caused no reduction in lesions beyond that caused by addition of juice of healthy plants. Thus, no evidence was obtained that neutralizing antibodies were present in the recovered plants. In evaluating these data, however, it must be remembered that the

treatment used to destroy virus or otherwise remove it from recovered-plant extract may likewise have removed antibodies, if such were present.

It is clear that present theories are inadequate to account for acquired immunity from plant virus diseases. While later study may show that antibodies play a part in this immunity, it seems more likely that the defense mechanism is of an entirely different nature.

Summary

To sum up briefly, the data accumulated during the past decade show conclusively that plants infected with certain virus diseases develop a defense reaction beyond the natural immunity inherent in the plant. There are some virus diseases from which plants recover and become refractory to reinfection. The immunity that ensues is of the carrier type, since the plant continues to harbor the virus. In many instances—in all instances so far studied—plant cells infected with one strain of a virus become immune from infection with a closely related strain. In this case, the immunity is usually of the chronic-disease type, since the plants lose neither the virus nor the symptoms produced by the virus. In both types of immunity, the nature of the defense mechanism is unknown. The nature of the mechanism is of importance not only with respect to plant virus diseases but also in the general field of immunity as a whole, for even in animal diseases the exact nature of the defense reaction is by no means solved. It is to be hoped that further studies on plant virus immunity will at least indicate the byways along which we need to go in pursuit of this elusive question.

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[3] For a fuller discussion of acquired immunity from plant virus diseases and for a more complete bibliography, see W. C. Price, "Acquired Immunity from Plant Virus Diseases," *Quart. Rev. Biol.* (in press).

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LOCAL AND GENERALIZED DEFENSE REACTIONS IN ANIMALS[1]

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IN reply to the question "What do you understand by 'injury' and the mechanisms of defense against it?" the students of the various biological disciplines give distinctly different answers. Some of the defense reactions in plants have just been discussed by the previous speakers. The biologist studying protozoa would probably discuss their reactions to starvation and poisons and predatory animals. The pathologist would reply in terms of parasites, inflammation, immunity. And further diversities of content appear in the answers of physiologists, biochemists, psychiatrists and the students of social organization.

One may say in general terms that anything which tends to interfere seriously with the local or general equilibrium in an organism tends to injure it; that defense is the reaction of the organism to ward off an injury or prevent its spread; and that repair is the attempt of the organism to return toward the state before injury. But as soon as we try to delimit these terms more closely, we encounter the almost universal difficulty of making consistent definitions of biological concepts. This results primarily from the extremely closely graded series and their overlapping of biological forms and processes.

Link encountered a similar difficulty in attempting to distinguish health from pathic states: "Sooner or later, in the course of its life cycle, events may and usually do occur within an organism with more or less frequency during which its reactions to internal and external stimuli

[1] Read in slightly abbreviated form at a symposium of the American Society of Naturalists in joint session with the American Society of Zoologists, the Botanical Society of America, the Genetics Society of America and Section N, the American Association for the Advancement of Science, at Columbus, Ohio, December 30, 1939.

exceed the limits of easy tolerance or even of its absolute adaptive capacity." My discussion will be centered in large part on those processes which he encloses in the phrase "easy tolerance."

In addition, the analysis of defense reactions has been confused by the introduction of mystical and anthropomorphic concepts and of teleological interpretations, as well as by the refusal of many biologists to admit that adaptiveness, even though explainable in physical and chemical terms, is characteristic of living reactions.

Living organisms are the seat of a continuous interplay between a wide variety of stimuli and the reactions which they call forth. Some of the stimuli arise within the organism, while others come from its environment and impinge upon it. When the quantity or quality of these stimuli or the reactions to them exceed certain limits, injury results.

The exogenous stimuli may be divided roughly into living and non-living. The living ones are primarily the lower forms of life which are potential parasites, saprophytes or commensals. For most animals, the first of these is the most important from the point of view of defense mechanisms. The non-living exogenous stimuli fall into three main groups: (1) mechanical, which includes local or generalized pressures that may result in trauma; (2) forms of energy, as temperature changes in environment, radiant energy, electricity; (3) chemical—this group includes the foodstuffs, water and gases taken into the body as well as other materials ingested or administered as drugs and poisons. Some of these substances cause injury by their absence (vitamin or other food deficiencies).

The endogenous stimuli are to a large extent concerned with the development of the organism and its functioning in the developed state; the reactions to them are merely expressions of the animal's activity as a living organism and are concerned with the maintenance of optimal conditions for life under a given environment. This includes

those mechanisms which act to keep the internal environment constant.

It is obvious that for any given individual at a given physiological level, different intensities of the same stimulus would produce different results. Further, the same stimulus administered to the same individual at different physiological levels would elicit still different results. Such differences become apparent when a population of similar individuals, each of which is at slightly different reactive levels because of individual heredity or previous experience, is exposed to the same stimulus of given intensity. In this case, variations in reaction will appear, although for most of the individuals the results are likely to fall within certain relatively close limits.

On the other hand, a given stimulus when applied to a variety of different species may call forth an extreme variety of effects, ranging from very intense reactions to those which are not detectable.

Stimuli may act over a long period; they may be repeated from time to time and call forth the same or different reactions. Stimuli may act locally or over the whole organism calling forth local or generalized reactions, or both.

A break in the mechanism by which a normal stimulus is mediated may result in injury. Or, the reacting mechanism may be insufficient to cope with a stimulus of greatly increased intensity and again injury may result. Hence, many physiological reactions may gradually merge into pathological ones. Is there any way of delimiting them?

It may be helpful to view this problem as exemplified in three experiments, involving different reactive mechanisms in which a gradual increase in the strength of the stimulus is applied. These will be presented in somewhat oversimplified form. A good example is the maintenance of the acid-base balance in the mammalian blood stream. If a small amount of acid is added to the blood stream, the alkali reserve falls but the pH remains practically the same because of the buffering action of the blood. Is this

a defense reaction? If much more acid is added continuously to the blood, a point will soon be reached when the alkaline reserve of this tissue is greatly diminished and other mechanisms will be called into play, as hyperventilation, increased acid elimination, mobilization of alkali from the other tissues. Is this stage injury and are the resulting processes defense reactions? Or must we wait until even more acid has been added to the blood in our experiment so that the subsidiary mechanisms likewise fail and the animal becomes manifestly ill and will die if the process is continued? Between each of the main stages in this experiment there is a continuous series of intermediate minor ones. Where should the line be drawn?

Let us consider another type of upset in function—the case of a diet deficient in phosphate. As long as the lowered phosphate absorption is sufficient, in the presence of large amounts of calcium, for the calcium-phosphate product to equal 40 mg/100 cc of blood, there is no injury in so far as bone is concerned. But if the amount of phosphate is decreased still further and the product falls below this figure injury will result, especially if extra need for phosphate becomes apparent as in pregnancy, and the body mobilizes phosphate from bones, or, if new bone is laid down it will be insufficiently calcified.

Still another type of experiment: Let us imagine that a very few bacteria penetrate an epithelium and reach the underlying tissue. If the cells at that point destroy the bacteria, there is no injury to the organism as a whole, even if some of the cells involved die. If more bacteria, or bacteria of greater virulence are introduced, the local cells will not be able to destroy the bacteria and other mechanisms will be called into play. This would be a reaction to injury.

Accordingly, for the ensuing discussion I shall consider the organism as being continuously exposed to exogenous and endogenous chemical and physical stimuli, many of which will be living biological systems or their products. If the stimuli are of such intensity that the mechanisms

immediately available at their site of intrusion can incorporate them into the complex interactions of the organism without disturbing it further, we may speak of reaction to stimuli of this strength as physiological. If the same stimuli are of greater intensity so that the mechanisms immediately available are insufficient and the organism must call on other mechanisms, I shall speak of these instances as injury. Or, in different words, defense may be conceived as consisting of a series of mechanisms of which the first line would be immediately available. If these are insufficient to cope with the stimulus, secondary mechanisms come into action. That is, defense consists of successive lines of reserves which become active in series after the previous defense mechanisms prove inadequate. If all of these become exhausted, the animal dies.

We may thus distinguish physiological and pathological reactions to stimuli, with the realization that these are merely extremes connected by a continuous series of intermediate stages. Somewhere between the two a limit may be set up, although few will agree on just where it should be placed. But if such a limitation is not made, it becomes impossible to speak of local and generalized defense mechanisms without including the entire physiological economy of the organism. What is meant by "immediately available" will have to be defined for each type of potentially injurious stimulus and each site of its action.

Because of their great variety it is impossible to discuss here the means used by the body in its defense against the host of injuries to which it at times is subject through increases in its endogenous stimuli or through failure of the mechanisms normally mediating them. Accordingly, I shall not consider psychic stimuli and psychic injury, or instincts, or hormone and metabolic unbalance, or hereditary defects, or the whole series of reactions by which organisms attempt to avoid injury through instinctive and volitional movements and defense weapons. Nor can I consider the defenses of the body against possible injury from many types of exogenous stimuli. For in all these cases the first lines of defense are as numerous as the

stimuli which they mediate when the dosage is not injurious.

I shall limit the discussion to an analysis of the reaction of the vertebrate and especially the mammalian body to the local and general introduction of several types of foreign substances. I shall point out how these animals, after being exposed to such an injury, are so changed that they react in different fashion to the reintroduction of the same materials.

The bodies of the vertebrates (and higher invertebrates) are composed of four main groups of tissue: (1) the epithelia which cover the body, line its cavities and form its glands; (2) the connective substance which forms the blood and blood-forming tissues, a large part of the vascular system, bone and cartilage and connective tissue between all other tissues; (3) muscle and (4) the nervous system.

As the covering epithelia separate the interior of the body from the external world, all exogenous stimuli must pass through them; they thus act as a barrier which at times is an important defense mechanism. In general, however, epithelium, nerve and muscle have but limited possibilities for defense; if they are injured sufficiently seriously they die and the nearby vascular connective tissue reacts to the injury. Eventually the damaged epithelium may be regenerated, although muscle and nerve display but limited regenerative potentialities.

REACTION TO LOCAL INJURY

The reaction to local stimulation will depend on the nature and amount of the stimulus and the defensive capacities of the local tissues. If the stimuli are of such weakness that the local mechanisms are sufficient to neutralize them, injury will not result. But if the stimulus is applied in increasing doses, a point will be reached which exceeds the capacity of the local tissue to prevent injury and secondary mechanisms of defense become effective. Such local injuries usually result in death of cells or mechanical interruption of histological continuity. As a

result of the injury, a complex series of changes occurs at that point which tends to localize the injurious stimulus, neutralize it and subsequently helps to repair the damage done. Most of this localizing reaction is carried out by the connective tissue, blood, blood-forming tissues and blood-vessels. A part of these reactions constitute the ill-defined inflammatory process. In general, it is much the same in all tissues (with a few exceptions such as the cornea, cartilage and central nervous system) and after most types of stimuli causing the injury. Although the process is fundamentally the same, it varies to the extent that different stimuli produce slightly different results and the site of the reaction will exert slight, local modifications of the process; for example, inflammation in the lungs and in the loose connective tissue.

The salient features of the simple inflammatory reaction are: First, it is a non-specific defense mechanism. Second, it is marked by the accumulation in the injured area of vast numbers of phagocytes which actively destroy part or all of the noxious material. Only a small part of these phagocytes existed in the tissue before it was injured; the circulating blood brings the great majority of them to this area as actual and potential phagocytes which arose in the blood-cell-forming tissues. Third, chemical substances pass from the blood into the injured tissue; this is not very important in most simple inflammations, although it is an important part of the specific inflammatory processes in natural or acquired immunity.

The failure of the inflammatory process to localize stimuli completely may be harmful as well as beneficial from the point of view of the animal; but in all cases it involves the whole organism in the reaction to local injuries. As we shall see, the failure to localize parasites and foreign proteins has a very important effect on the organism for, as a result of their passage into the rest of the body, reserve lines of defense react and the host becomes specifically immunized to the particular foreign agent.

The local defense reactions to aseptic injuries (those

from which parasites are absent) are the simplest to study and give a general picture of the process. The modifications that may be caused by the presence of invading parasites will be more apparent if the basic sequence of events is pointed out first. In aseptic inflammation in most instances all the injury is done at once. This is rarely the case in septic injuries in which the parasites usually multiply and continue to stimulate the defense reaction.

The reaction that follows a cut into the subcutaneous tissue, or the introduction there of a salt of a heavy metal or of a foreign protein is essentially the same in the several instances. Let us follow the local consequences of the subcutaneous injection of a small amount of a slightly irritating, vital dye into a rat. The use of this material is particularly helpful in that it aids us to discriminate between certain of the cell types involved in the reaction following its introduction. The first change after the dye is injected will be a slight swelling of the connective tissue due to the dyestuff. During the first hours of the reaction the capillaries and venules of the affected area undergo a marked dilatation. They show a marked increased permeability, and blood plasma flows into the area.

The next change in the tissue is the movement of cells from the blood into the injured area. Great numbers of heterophil granular leucocytes and of non-granular leucocytes (lymphocytes and monocytes) migrate from the vessels into the injured area which is now edematous through the accumulation of blood plasma. The heterophil leucocytes and the monocytes are each capable of ingesting certain kinds of finely divided particulate matter, while the lymphocytes rarely show this power. The migration of leucocytes of all types continues to a marked degree for the first eight to ten hours, after which it tends to diminish. It is necessary to stress the movement of the leucocytes and the liquid parts of the blood from the vessels because they represent important, easily mobilizable forces which act in defense. After their migration from the vessels, the lymphocytes and the monocytes begin to hypertrophy.

This is indicated at first by enlargement of cytoplasm or nucleus or both, with the result that a great variety of cell forms is present; these do not conform to any of the cells of the blood or normal connective or blood-forming tissues (with the exception possibly of the omentum).

During the next few hours these cells continue to hypertrophy, and at eighteen to twenty-four hours they begin to store small amounts of the dyestuff. This process of hypertrophy continues and by the end of the next twenty-four hours the inflamed tissue is crowded with masses of newly developing macrophages which have come from the hypertrophy of the blood, non-granular leucocytes and a few local macrophages; at the end of four days these new macrophages can not be distinguished from the relatively few macrophages of local origin in the inflamed area. All of the macrophages now contain large amounts of the dye.

With the start of about the fifth day, large numbers of the macrophages begin to develop into fibroblasts. During this transformation the cells become outstretched and larger and gradually lose their content of dye and of phagocytosed cellular débris. In the course of the next week the large numbers of fibroblasts now present begin to lay down collagenous fibers. The inflammatory reaction is subsiding.

If, instead of being produced by the injection of a vital dye as above—or by that of a foreign protein or salt of a heavy metal—the injury had been the result of a sterile knife wound, a defect in the tissue would have resulted. This would have been filled at first by plasma, which would then clot. The granular and non-granular leucocytes would wander into this clot. Here the granulocytes would die and the non-granular cells would hypertrophy into macrophages. Next to enter this area would be macrophages, fibroblasts and blood-vessels from the surrounding tissue. Eventually, the macrophages from these two sources in the defect develop into fibroblasts and produce dense masses of collagenous fibers; this is the scar.

What are the changes resulting locally from the injection

of bacteria and most other parasites into the tissue? The details of the reaction will vary somewhat with the virulence, type and numbers of the parasites and whether the reaction occurs in a normal or immune host. In the normal host, in most cases, the inflammatory exudation will be qualitatively the same as described above and the same cellular transformations will occur, but the percentage of granular leucocytes will be higher. Depending on the factors of virulence, etc., mentioned above, the parasites will or will not be destroyed by the macrophages and granulocytes ingesting them. This type of injurious agent differs from the non-living ones in its ability to multiply rapidly and in many cases to give off toxic substances which must be neutralized to prevent further injury to the host. In most cases of local bacterial injury, the migration of cells from the blood stream is of greater intensity and lasts longer than in aseptic injuries.

What has been accomplished as the result of this complex inflammatory reaction? In each of the above cases, the dead cells resulting from the injury have been cleared away. The defect resulting from the knife wound has been filled with scar tissue. Although much of the vital dye has been localized, most of it has diffused beyond the site of the injection and has entered the circulating blood, whence it is removed by the macrophages in the liver, spleen, etc., so that a few repetitions of the injection make it possible to "stain" the macrophages of the whole animal vitally. In the case of the foreign protein, too, only some of the material is destroyed locally, for some of it can be detected later in the blood stream and it produces changes in other organs. Although great numbers of bacteria and other parasites are destroyed in the inflamed areas, some of them or their products gain access to the general circulation, whence they tend to be removed by the macrophages, especially those of the spleen, liver, bone marrow, while those which have entered the lymph stream tend to be taken up by the macrophages of the nearest lymph nodes. This means that further lines of defense come into activity. A notable example of the incomplete localization

of invading parasites by inflammation in the invaded tissue is shown in malaria. Here a relatively insignificant inflammation ensues, the parasites do little damage, but manage to reach the blood stream, where they multiply. This is especially striking in that malarial parasites reproduce slowly as compared with most bacteria.

One of the important generalized reactions to a local introduction of parasites is the maintenance of the leucocyte level in the blood stream. We have seen that there is extensive migration of the white blood cells into the local area of injury. The movement of these cells out of the blood stream calls upon the bone marrow and lymphatic tissue reserves, and extensive changes take place in these blood-cell-forming organs in order to maintain adequate numbers of their progeny in the circulating blood. This is especially pronounced in the lymphatic tissue, where the lymphocytes are normally stored in great numbers. These cells move rapidly into the veins or into the afferent lymphatics and thence into the blood stream. This temporary depletion is quickly followed by a wide-spread multiplication of lymphocytes and the lymphatic tissue increases in amount. This is exemplified by the well-known "acute splenic tumor" of many acute infections. The demand for lymphocytes may be so great that even this rapid multiplication of them is insufficient and the lymph nodes and spleen then become depleted of their free cells and greatly reduced in size. With the subsequent destruction of the invading parasites, recovery ensues and the reserve supply of lymphocytes is again built up. These reactions can take place readily and in a relatively short time. This lability of structure of the lymphatic tissue is an important one, for it demonstrates that the lymphatic tissue all over the body is a source of easily mobilizable cells which can migrate into local inflamed areas and hypertrophy into macrophages.

It has been claimed that part of the localizing effect of the inflammatory reaction is due to clotting within the local lymphatics, thus preventing the dissemination of the ex-

traneous materials from the inflamed area. Most of the recent work does not agree that the lymphatics are blocked. In any event, it is clear that in the described cases of injected vital dyes and foreign proteins, the ensuing inflammatory reaction is insufficient to localize the stimulus.

REACTIONS TO GENERALIZED PARENTERAL STIMULI

We have just seen that substances ranging in character from vital dyes to proteins and parasites are often not localized completely by the mechanisms which come into play at their sites of introduction. These foreign substances consequently become generalized stimuli and elicit further defense reactions which tend to neutralize and destroy them. The type of generalized defense reaction will depend to some extent on the kind of substance introduced.

The host of pharmacodynamic materials acts on such a variety of tissues and organs that it is impossible to consider them in this brief general discussion. I shall consider only those types of substance which were described above as causing local injury.

Innocuous foreign particles, as colloidal carbon, are removed quickly from the blood stream by the macrophages in spleen, liver and bone marrow.

If the foreign materials are proteins or bacteria or other living parasites and are disseminated over the body by the blood stream, the resulting reaction again will depend on the type, virulence and number of the invading organisms and the degree of natural immunity of the host. With the exception of certain parasites, as those of kala-azar, which live in macrophages, organisms of low virulence and foreign proteins will be quickly removed from the blood stream and digested by the macrophages in the spleen, liver and bone marrow. In this case the reaction involves the first line of defense only, and injury, as I have defined it, does not occur. But if the parasites are of greater virulence, the macrophages, or at least some of them, will be destroyed, the parasites will begin to multiply rapidly and great numbers of new macrophages will be mobilized.

This is a reaction to injury and will occur to a small extent through the mitosis of local macrophages, through the mobilization of macrophages from the reticular cells, and largely through transformation of lymphocytes into macrophages. This process, if the demand is great enough, will cause striking changes of depletion and, eventually, overproduction of lymphocytes in the lymphatic tissue. If the parasites are present in overwhelming numbers, or are of such a nature for a particular host that the defense of the host is insufficient for the production of adequate numbers of macrophages, the animal will succumb.

One of the most important consequences of this phagocytic reaction of the body to the introduction of foreign proteins and organisms is the production of substances called antibodies. The antibodies have the ability to precipitate specifically these foreign materials (and to agglutinate them if they are cells) *in vitro*. They have the ability to render these particular foreign substances more readily phagocytosed *in vivo*. This development of antibodies is a part of the process of active immunization. As a result of this process the defensive mechanisms are specifically increased, with the consequence that the organism has an increased capacity for reacting quickly and effectively to the local or generalized reintroduction of the same foreign material.

This increased defensive capacity of the animal persists for varying periods of time and rests primarily on: (1) localization of the irritant (precipitation, agglutination) and (2) increased activity of phagocytes (opsonization).

The Local Reaction to the Local Reintroduction of Foreign Substances

Here again the reaction varies with the type of substance introduced, whether it is the same as that injected the first time, and whether or not it enters the same or similar tissue area as the first time.

(1) The reinjection of most non-protein materials into the same area as previously injected (after a sufficient time has elapsed for healing to have taken place) results in an

inflammatory reaction of the same intensity and quality as after the first injection. In this case there seems to be no change in the first lines of defense.

(2) After reinjection of the same antigen, a much more violent reaction usually ensues. This depends on whether antibodies had developed against this particular antigen. There has been a long discussion as to whether antibodies develop locally or generally or both. The majority of recent opinions is that both sources function. In the case of reintroduction of the antigen into a tissue area similar to but not identical with that involved in the first inflammation, it is obvious that the antibodies which appear with the inflammatory exudate come from other tissues by way of the blood. No matter whether the antibody has been produced locally or generally, it appears in the reinjected area of local inflammation and there unites with the antigen. In general, this antigen-antibody union results in an intense inflammation and cell death.

(3) If a living parasite is injected into the site of a previous non-specific inflammation, the macrophages aggregated at that point together with those resulting from the new inflammation enable the host to overcome the injected bacteria very quickly if they are of low virulence. That is, the immediate defense capacity of the tissue is increased because of its previous experience and a stronger stimulus is necessary to produce injury. But if the bacteria are of high virulence (as in the case of the introduction of pneumococci into rabbits by Cannon and co-workers) previous non-specific inflammation does not materially increase the local defense toward this infection.

If, however, the area had previously been injected with the same bacteria, the reinjected organisms are usually destroyed exceedingly quickly and the infection is localized. In this case the defense capacities of the tissue had been greatly enhanced by the presence of specific antibody (opsonin) which enables the macrophages remaining from the previous injection and those arising from the new stimulus to be much more effective phagocytes. The anti-

body, by agglutinating the bacteria, also tends to prevent their dissemination.

(4) The local injection of a parasite in an animal which had a previous general infection with this parasite usually results in a very rapid local destruction of the invader if antibodies have been produced in great amount during the first generalized infection. However, there are some infections in which the presence of great amounts of antibody fails to help protect the host completely. On the other hand, in those infections in which a high degree of immunity develops with the production of only small amounts of circulating antibody the local reaction to reintroduction of the organism does not always result in local destruction of the invaders. This is clearly seen in the attempt by Professor Taliaferro and myself to observe the local reaction to infections of malarial blood in normal and immunized canaries and monkeys. In the two sets of normal and immune animals the local reaction appeared identical. Here the local reaction was inadequate for the localization and destruction of the parasites, and the more generalized defense reactions came into play and prevented reinfection.

From these experiments with local reintroduction of foreign materials it is apparent that the new inflammatory reaction differs from that after the first injection. In these animals, as a result of the previous experience with the same foreign protein or parasite, specific antibodies developed against the foreign materials so that when they are reintroduced, the inflammatory reaction has become a highly specific and effective means for the localization and destruction of the foreign material. In such animals, dosages of a given bacterium which would have killed normal animals very rapidly are quickly destroyed.

Reactions to Repeated Generalized Parenteral Chemical Stimulation

In the case of certain drugs, such as cocaine, the organism reacts to repeated doses of the substance in the same fashion each time so that the dosage of the drug need not be increased to produce a definite reaction. In the

case of repeated introduction of morphine, on the other hand, the body develops a progressive ability to conjugate the drug and render it ineffective. This means that the dosage must be continually increased to satisfy the craving which develops. In this case the body develops a defense against the possible harmful action of the drug; this is called an increase in tolerance and does not have the same basis as antibody production.

With repeated administration of a foreign protein, the body develops great amounts of antibodies to this substance. The generalized (intravenous) administration of the protein in such an immunized animal results in different reactions which depend primarily on the animal species. At some stages in the immunization process, animals of most species become hypersusceptible to the introduction of the foreign protein and suffer reactions of varying degrees of severity consequent to the union of antigen and antibody. In some species it is almost impossible to elicit this hypersensitive phase of immunization.

In the case of reintroduction of the same parasite, the macrophages all over the body ingest them extraordinarily rapidly. This is effected in part through the increased mobilization of macrophages following the first infection, and in even greater numbers through the presence of specific opsonins (antibodies) which enable the macrophages to be much more effective phagocytes. This part of the immune reaction is highly specific for the particular parasite.

An important part of this defense reaction is the mobilization of the lymphocytes of the lymphatic tissue with a subsequent hyperplasia of this tissue. This produces an easily mobilizable and transportable supply of lymphoid cells which can readily turn into macrophages. It has been shown in such different infections as those due to malaria and *B. monocytogenes* that the qualitative changes in the lymphatic tissue are much the same in the first infection and in the reinfection but that the sequence of events starts earlier and is much more rapid and intense in the immune animal.

These recent studies throw light on the so-called mesenchymal reserves of the body. These, in the adult organism, are reserve sources for (1) erythropoiesis, (2) leucopoiesis, (3) phagocytosis, (4) mechanical support by connective tissue, bone and cartilage. It is not possible to enter in great detail here into these complex problems. I have shown elsewhere that the mesenchymal reserves of the adult animal consist of two types of cells, (1) the direct descendants of the fixed mesenchymal cells which appear in the blood-forming tissues and along the capillaries as non-phagocytic reticular and perivascular cells, respectively, and (2), as free mesenchymal cells, the lymphocytes (also called hemocytoblasts). These occur to a limited extent in the bone marrow and in vast numbers in the lymphatic tissue of the lymph nodes and spleen, whence relatively small numbers of them continuously gain access to the circulating blood. Because of the close functional relationships between the lymphocytes and macrophages in the reaction to single and multiple injections of foreign proteins and parasites, Taliaferro and Mulligan have proposed the term lymphoid-macrophage system.

Although most of what I have said deals with the phenomena of inflammation and immunity, it must be kept in mind that these are not the only or the main mechanisms of defense. Although these processes result from certain types of injury and serve in many cases to neutralize or to aid in preventing the spread of the injurious agent, they are only part of the defensive mechanisms of the body. Inflammation itself is a non-specific reaction of the body to many types of local injury, usually those involving death of cells. It obviously is not "the" local defense reaction.

Summary

I have tried to show that a great variety of stimuli are constantly acting on the animal body and that diverse mechanisms act to incorporate them into the functioning organism. If for reasons of quantity or quality, the mechanisms reacting to these stimuli are inadequate, injury results. In the gradation between physiological and patho-

logical reactions, I have set an arbitrary limit based on the criterion of whether the mechanisms immediately available at the site of action of the stimulus are adequate to neutralize the stimulus.

The action of many types of stimuli results in local injury and the immediate reaction of the animal is by inflammation. Through this non-specific reaction, blood plasma and large numbers of phagocytes accumulate in the injured area. The blood contributes the vast majority of these through its granulocytes—which are relatively specialized phagocytes—and through its lymphocytes and monocytes which soon hypertrophy into macrophages. The phagocytes remove or neutralize much of the foreign material.

The simple inflammatory reaction is often an incomplete barrier to the extension of the harmful agent. As a consequence, further defense mechanisms of more generalized type become active. If the injurious agents are foreign proteins or parasites, specific antibodies are formed against them—presumably by the macrophages—as part of the process of immunization.

Simple inflammation differs from immune inflammation, for in the latter case the organism through its natural or acquired immunity reacts specifically and in most instances very efficiently in the neutralization of noxious factors.

The generalized reintroduction of a foreign protein or parasite into an animal which is specifically immunized results in most instances in a relatively quick neutralization of the noxious agent through the efficient interaction of macrophages and antibodies. In many cases, particularly if the dosage of the stimulus is small, these reactions may be so efficient that it may be difficult to demonstrate the reactivity of the host. Here it is apparent that a stimulus, which in a non-prepared host would have required an extensive series of defense mechanisms, is now overcome by the first line of defense of the immunized animal.

The lymphatic tissue acts as a mesenchymal reserve in

the simple and immune, local and generalized reactions by furnishing large numbers of lymphocytes which readily turn into macrophages.

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A RELATION BETWEEN BREED CHARACTERISTICS AND POOR REPRODUCTION IN WHITE WYANDOTTE FOWLS[1]

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IT is to be expected that originators of new breeds of domestic animals, in their efforts to establish breeds differing from those already existing, will occasionally adopt as a breed characteristic some exceedingly undesirable mutation. In a recent review (Hutt, 1934) there were discussed 31 lethal mutations in domestic animals. Of these no less than five had been incorporated as breed characteristics to produce Dexter cattle, Creeper (Scots Dumpie) fowls, the hairless dog, Crested ducks and the grey Tzourcana and Shiraz sheep. To this list can now be added two more such cases. In the Dark Cornish (Indian Game) fowls, the more short-legged birds, according to Landauer (1935), are heterozygous for a lethal mutation different from that in Creepers. Lienhart (1937) found that in the so-called rosy Gier pigeon a sex-linked lethal gene has been incorporated to turn the unwanted ashen-headed birds into the creamy-headed type which the fancier desires.

Evidence for another case of low reproduction associated with breed characteristics is presented in this paper. Poultry breeders have long complained of White Wyandottes as being inferior to other breeds in their capacity to reproduce, but actual proof of this contention has not hitherto been advanced. Experimental demonstration of breed differences in reproductive capacity requires: (1) that the breeding birds compared should come from several different strains of the breeds to be compared; (2) that they be random samples with respect to their abilities to reproduce; (3) that the environmental, dietary and mating conditions be practically identical for all birds; (4) that the eggs be incubated under identical conditions; (5) repetition

[1] Number 10 in the author's series "Genetics of the Fowl."

of the test for several years, with changing stock, till conclusive results have been obtained.

The Data

All these requirements have been met by the National Official Pedigree Breeding Station operated from 1929 to 1935 by the Lancashire Utility Poultry Society at New Langton, Lancs., England, and also by the Northern Ireland Pedigree Poultry Breeding Station at Hillsborough, County Down, which has been conducted since 1933 by the Agricultural Research Institute of Northern Ireland.

The object of both stations was to provide official supervision over the pedigreeing of chicks hatched from fowls which had previously qualified, by laying 200 or more first-grade eggs in an official laying test, for registration by the National Poultry Council. At each station housing, feeding, management and incubation were alike for all birds. Records were kept of the numbers of eggs incubated, infertiles, dead-in-shell, chicks hatched, crippled and weak chicks, losses during rearing, culls eliminated and of healthy chicks reared. These are summarized in the annual reports of the two stations. The plan of operation was practically the same in Northern Ireland as in Lancashire.

Nowhere in the published reports of these enterprises is there any suggestion that a comparison of breeds was contemplated. However, their records do provide exactly the data needed for comparisons of breeds with respect to efficiency of reproduction.[2] The analysis in this paper is restricted to White Leghorns, Rhode Island Reds and White Wyandottes, which together comprised about 90 per cent. of all birds entered. To emphasize the adequacy of these birds as samples of the breeds compared, their sources for representative years are given. In 1931 pedigreed progeny were produced at the Lancashire station by

[2] The author is indebted to Mr. R. W. Hale, secretary of the institute, for information facilitating analyses of the records from Northern Ireland, and to Mr. E. Collier, secretary of the society, for records and information concerning the Lancashire station.

Leghorns from 24 different farms, Rhode Island Reds from 17 farms and Wyandottes from 18 farms. In Northern Ireland in 1936 Rhode Island Reds were sent in from 28 different farms, White Leghorns from 12 and White Wyandottes from 30. All the birds at this station came from Northern Ireland, whereas in Lancashire the birds came from all over England and Scotland, but, with the exception of a single entry in seven years, not from Ireland. The data from both stations thus make the sampling of the breeds much more adequate than could those from either station alone. Altogether about 15,000 eggs from each breed are involved.

TABLE 1

SUMMARY OF REPRODUCTION IN THREE BREEDS*

Breed	Number eggs incubated	Infertile Per cent.	Dead in shell Per cent.	Chicks hatched Per cent.	Weak chicks Per cent.
Lancashire (7 years)					
White Leghorn	12,521	20.0	19.1	64.8	6.5
Rhode Island Red	9,887	17.0	18.6	67.6	5.3
White Wyandotte	10,966	31.4	21.6	53.8	11.8
Northern Ireland (5 years)					
White Leghorn	2,271	10.7	25.4	66.8	8.2
Rhode Island Red	7,236	9.0	20.0	72.3	3.9
White Wyandotte	4,842	19.3	21.4	63.4	8.1

* The percentages are based as follows: Infertile eggs—upon the numbers incubated; dead-in-shell—upon the numbers left in after removal of the infertiles and dead embryos on the eighteenth day; chicks hatched—upon the numbers of eggs incubated; weak chicks—upon the numbers of chicks hatched.

To save space the actual numbers of infertile eggs, chicks hatched, etc., for each breed each year are omitted and a summary is presented (Table 1) showing the percentages for the critical factors by which the efficiency of reproduction can be measured. This covers the seven years during which the Lancashire station was operated and the five years from 1934 to 1938 for Northern Ireland. The data for 1933 at the latter station are omitted because the numbers in that year were small and the conditions of incubation and candling were not the same as in later years.[3]

[3] Infertility appears somewhat higher at the Lancashire station than it really was because candling there was done only on the eighteenth day, at which time all eggs recognized as containing dead embryos were lumped with

Analysis of the Data

In determining the significance of the differences between breeds, tests for heterogeneity showed that there was too much variation from year to year to permit all the data for any breed from one station to be lumped together as a homogeneous sample to which the standard error of a percentage could be applied. Environmental conditions evidently varied enough to raise the hatchability for all breeds in one year and to lower it in the next. Accordingly, comparisons were made by Student's pairing method so that at each station one year's figures for any one breed were compared separately with those for each of the other two breeds in that same year. For example, in determining the difference between Wyandottes and Leghorns in fertility there was a series of seven comparisons in Lancashire and five in Northern Ireland. In Table 2, where the mean differences are shown, the values for p give the probabilities that differences as great as those observed might have arisen by chance.

TABLE 2
Differences Between Breeds

Comparison	Infertile		Chicks hatched		Weak chicks	
	Mean difference	p	Mean difference	p	Mean difference	p
Lancashire						
W. Wyand. —W. Leghorn .	+10.6*	.014	−12.5*	<.01	+5.1*	<.01
W. Wyand.—R. I. Red	+13.2*	<.01	−11.8*	<.01	+6.7*	<.01
R. I. Red—W. Leghorn ..	− 2.7	.29	+ 1.1	.71	−1.6	.18
Northern Ireland						
W. Wyand.—W. Leghorn .	+ 9.7*	<.01	− 4.0	.48	−0.3	.82
W. Wyand.—R. I. Red	+10.3*	.019	− 8.2	.07	+3.8*	.016
R. I. Red—W. Leghorn ..	− 0.7	.63	+ 4.2	.28	−4.1*	<.01

* Statistically significant differences.

The number of chicks hatched per hundred eggs incubated was consistently lower in Wyandottes than in the other two breeds. The differences of 11.8 and 12.5 per cent. were statistically significant in the Lancashire data,

the infertiles in a class called "unfertile." In Northern Ireland "addled" eggs containing embryos dying before the eighteenth day were classed with the dead-in-shell. This difference in procedure explains the differences between the two stations with respect to frequencies in these two classes.

but those of 4.0 and 8.2 per cent. in Northern Ireland were not. At the latter station the number of Leghorn hens was small, varying in the five years from 12 to 16. With such numbers, one or two poor sires could affect adversely the record of reproduction for the year. The Leghorn records fluctuated more than those of other breeds. In 1938, when the number of Wyandottes also dropped to 14, their eggs actually hatched better than those of both the other breeds. It is not surprising, therefore, that the breed differences in chicks hatched were not more significant statistically at this station.

The data in Table 1 show no consistent breed differences in dead-in-shell, although with the exception of the Irish Leghorns the Wyandottes were a trifle higher in this respect than the others. The factor affecting most the numbers of chicks hatched was the infertility, which at both stations was higher in Wyandottes than in the other two breeds (Table 2). The differences, varying from 9.7 to 13.2 per cent., were all highly significant statistically. Apart from this analysis the consistency of the differences

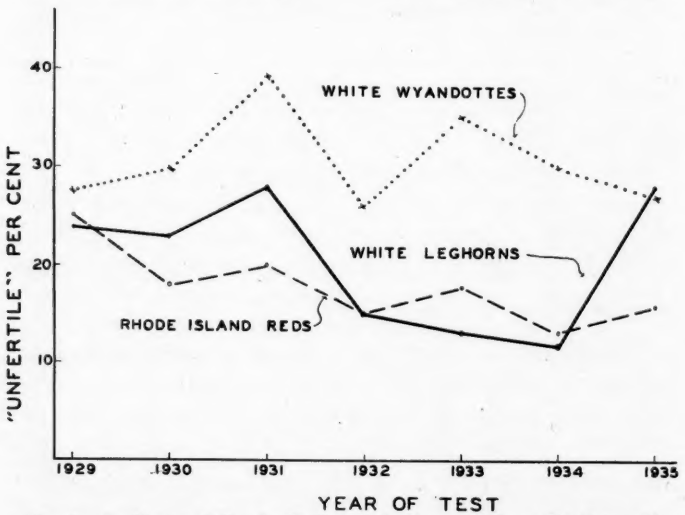

FIG. 1. Incidence of "unfertile" eggs in three breeds at the Lancashire station over seven years.

from year to year is convincing. Fig. 1, where data for the "unfertiles" at the Lancashire station are plotted, shows that the Wyandottes were consistently higher in this respect than both other breeds in every year except 1935, when the sample of Leghorns was too small to prevent considerable fluctuation.

The "weak chicks" include cripples and defective chicks of various kinds. In Lancashire the proportion of these in Wyandottes (11.8 per cent.) was higher than in the other two breeds by statistically significant differences. The consistency of these from year to year is shown in Fig. 2. Similarly in Northern Ireland there were significantly fewer weaklings among Rhode Island Reds than in Wyandottes, but the Leghorns had slightly more. This may have resulted in part from the smaller sample of the Leghorn breed at that station. It is noteworthy that the proportion of weak chicks was lower at both stations in Rhode Island Reds than in the other two breeds.

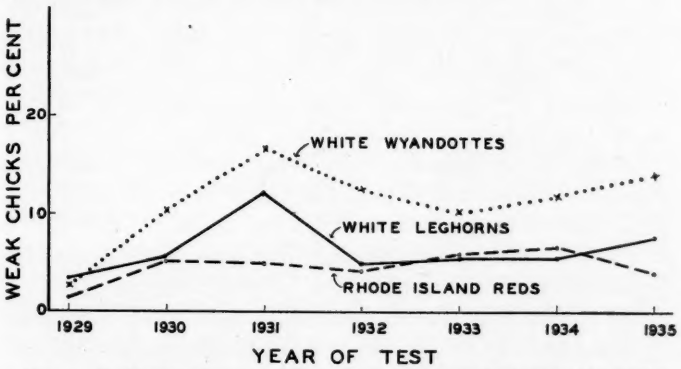

FIG. 2. Incidence of weak chicks in three breeds at the Lancashire station over seven years.

The White Leghorns and Rhode Island Reds did not differ significantly in any of the measures of reproduction, with the doubtful exception of weak chicks in Northern Ireland. This makes all the more striking the consistent and significant differences between these two breeds and the Wyandottes.

DISCUSSION

Something associated with the genetic constitution of the White Wyandotte is evidently incompatible with maximum efficiency in reproduction. Since neither station made a sharp distinction between fertile eggs and those containing embryos which died early, the question is still open whether the difficulty lies in failure of the eggs to be fertilized, or whether fertility is normal but early embryonic mortality unusually high. In eggs not candled till eighteen days of incubation, those in which embryos had died during the first two days might easily be classed as infertile. For that reason the data under discussion, though indicating that fertility is abnormally low in White Wyandottes, do not exclude the possibility of early embryonic mortality being equally responsible for subnormal reproduction in that breed.

No reason is apparent why a breed subject to low fertility should also have a high proportion of weak chicks. The differences reported may have resulted as much from preferences of the persons grading the chicks as from anything else, and, since the defects are not specified, the breed differences in "weak chicks" should probably not be considered as definitely established.

There are indications that some fowls infected with *Salmonella pullorum* tend to have more than average mortality among their embryos. The possibility of differential breed susceptibility to that organism being indirectly responsible for the differences in reproduction is eliminated by the fact that at both stations only birds proven by blood test to be free from that organism were admitted.

Since White Wyandottes are recessive white, it is possible that a lethal gene linked with that color, for which some evidence was found by Dunn (1923), may be a factor affecting reproduction in that breed. However, other recessive white breeds and varieties have not been subject to the same suspicion as White Wyandottes. In this connection it is of interest to recall the popular (but unconfirmed) belief among breeders of Shorthorn cattle that white heif-

ers are "shy breeders." Whether or not colored varieties of Wyandottes are as poor breeders as the white ones remains to be determined.

Rhode Island Reds seem the most efficient of the three breeds, so far as reproduction is concerned, since in that breed infertility, dead-in-shell and weak chicks are lower at both stations than in the other two breeds. However, the differences between Reds and Leghorns are in general much smaller than those between either of them and the Wyandottes and are less consistent.

Not all White Wyandottes are poor breeders. The fact that some males and females have good capacity to reproduce has led to considerable controversy over the reputed shortcomings of the breed, with some poultrymen stoutly maintaining that reproduction in Wyandottes is fully as good as in other breeds. It is evident, however, that the average for the breed is low. The condition responsible may be a pleiotropic effect of some gene, or genes, determining the characteristics of the breed, or it may result from genes linked with those making the breed. In either case it is as much of an inherited trait as are the rose comb and white color by which the White Wyandotte is distinguished.

Recognition of this fact may be responsible for the waning of the breed's popularity in the United States, but till recently it has been a favorite in the British Isles. Of all farms sending registered hens to the Lancashire station during its last three years, 1933–35, 30 per cent. had White Wyandottes. In Northern Ireland, 32 per cent. of all farms entering birds during the three-year period, 1935–37, sent that same breed. However, the writer has been advised by Mr. R. W. Hale that the proportion of Wyandotte entries in the official laying tests in England, Scotland and Northern Ireland declined from a high figure of 28 per cent. in 1932 to 17 per cent. in 1937. This probably indicates recognition of the breed's shortcomings. White Wyandottes make unexcelled table fowls, and strains capable of good egg production have been established. It is to

be hoped that the cause of the subnormal reproduction may be discovered and eliminated from a breed which is otherwise deservedly popular.

SUMMARY

Analyses of records of official pedigree breeding stations in Lancashire and in Northern Ireland show that the ability of White Wyandottes to reproduce is subnormal in comparison with White Leghorns and Rhode Island Reds. A total of about 15,000 eggs for each breed is involved, and the samples came from many different farms. The numbers of chicks hatched per 100 White Wyandotte eggs incubated were 12.5 and 11.8 less than for White Leghorns and Rhode Island Reds, respectively, at one station and 4.0 and 8.2 less at the other. These differences apparently resulted chiefly from infertility, though early embryonic mortality may also have been a factor.

The subnormal reproduction is attributed to the pleiotropic action of some gene, or genes, determining the characteristics of White Wyandottes, or to linkage of deleterious genes with those making the breed.

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SUBSPECIES, HYBRIDS AND SPECIATION IN DROSOPHILA HYDEI AND DROSOPHILA VIRILIS[1]

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INTRODUCTION

THE work on *Drosophila melanogaster* is too well known to warrant any general discussion of the value of Drosophila as a genetic tool. Yet from time to time there have been certain objections raised to the practicability of using this animal for studies on the nature of evolutionary processes. One early objection had to do with the very small size of Drosophila chromosomes. To-day the giant salivary chromosomes of the Diptera are recognized as better than large plant or amphibian chromosomes for cytological study. Their enormous size and intricate visible pattern have made possible the development of a new cytology at a level of analysis impossible with other material.

A second objection sometimes raised was that mutations in Drosophila were of the "bottle" variety. And indeed with the perfection of the x-ray technique for manufacturing mutations wholesale and the almost universal application of this technique by the majority of Drosophilists in a variety of experiments purporting to deal with the factors of natural evolution there may have been some reason for this criticism. We do not mean to minimize the importance of x-rays in studying the structure of the chromatin. But it has seemed a rather invalid assumption that x-ray changes are necessarily parallel in quality to

[1] The major part of the work herein reported was done while the author held a fellowship under the Rockefeller Foundation at the William G. Kerckhoff Laboratory of Biology, California Institute of Technology. The author wishes to thank Dr. T. H. Morgan and his staff for the generous use of facilities in that laboratory, Dr. Th. Dobzhansky for suggestions concerning study of hybrid sterility, Dr. M. Steggerda for material collected and especially Dr. A. H. Sturtevant for his generous help and sound criticism on matters dealing with taxonomy.

and merely differ in quantity from natural mutational processes. Recent evidence from several sources points to qualitative as well as quantitative differences in x-ray and natural mutational changes. However, it is now recognized, at least by those who have worked with natural populations, that many of these are full of mutations, and that in certain places one can collect in an afternoon Drosophila material containing hundreds of them. In fact, for time expended the method of collecting wild flies and breeding out mutations which they carry yields more workable auto-somal material than any x-ray technique yet devised.

A third objection to Drosophila was that there were almost no species and subspecies crosses possible in the genus, the early case of *simulans-melanogaster* hybrids reported by Sturtevant (1919) being presumably quite exceptional. As a matter of fact relatively recent and critical study of wild Drosophila material indicates that many interesting and significant stages in evolution at the subspecies level are extant in this genus.

The classical work of the Morgan school on the genetics *D. melanogaster,* the ingenious experiments of Muller leading up to the discovery of transmutation by x-rays and the subsequent careful analysis made possible by this technical tool, and particularly the epochal discovery by Painter of the significance of the morphology of salivary chromosomes form a firm foundation for the more extensive work which lies ahead. The writer wishes to emphasize this point as some biologists, even some Drosophilists, seem to think that the Drosophila field has been pretty well worked, except for a detailed extension of cyto-genetic and physiologico-genetic findings via the x-ray, salivary chromosome and transplantation techniques. Actually we have at hand the material for the intelligent investigation of the causes and course of evolution in one group. This material must be handled on a scale never before attempted and by combined field, laboratory and field-experiment methods yet to be developed. The nearest approach to such studies are those made by Dobzhansky and Sturtevant (1938) and Dubinin and his co-workers (1937).

We here present some data on subspecies and subspecies crosses in *Drosophila hydei* and *Drosophila virilis* and attempt to relate them to other known cases of hybridization in the genus.

A New Subspecies of Drosophila hydei

In June of 1938 our laboratory at Wooster received a shipment of Drosophila species from A. H. Sturtevant. This included four stocks of *D. hydei* collected in Yucatan, Mexico, by M. Steggerda, of the Carnegie Institution, and sent through Dr. M. Demerec to Sturtevant. On sending us this material Dr. Sturtevant called attention to the peculiar light thoracic pattern of these flies, differing from typical *D. hydei.* Dr. Steggerda has written that the material was collected either in Merida or Chichen Itza, Yucatan.

When the condition of these stocks permitted we made up many pair matings and reared some 100 F_2 cultures and examined these for mutants. Among the offspring of several of these there appeared an extreme abnormal abdomen, autosomal recessive and semi-sterile. This was the only mutant recovered by inbreeding and is one of the commonest types of mutation found in *D. hydei.* During this analysis several thousand flies were examined. As we have carried on extensive experiments of this type using wild *hydei* material from widely separated sections of this country and in several years we are in a position to know something of the normal variability of the species. The Yucatan fly was seen to differ in several morphological characters from true *hydei.* It has now been cultured in our laboratory for almost a year and continues to show this slight but constant ensemble of character differences. Furthermore, crosses with typical *hydei* stocks indicate that these characters are due to multiple factors and not to one or two gene differences. On these grounds therefore we are describing it as a new subspecies, *D. hydei yucatanensis.* There follows a taxonomic description. Only those characters in which it has been found to differ from *hydei*

hydei are included. The reader is referred to Sturtevant's description of *hydei* (1921) for an account of characters common to both subspecies.

TAXONOMIC DESCRIPTION, DROSOPHILA HYDEI SUBSP. YUCATANENSIS, SUBSP. N.

Differs from *hydei hydei* in having the dark brown spots around macro- and microchaetae of the thorax much reduced in size, giving a pale, bleached appearance. This character resembles a sex-linked mutant type known as "light," but is not that mutant. A median longitudinal dark band or stripe, including the region of the middle four or five acrostical rows of microchaetae, narrower anteriorly and best seen in young flies. Eyes larger than in *hydei hydei*, being intermediate to *hydei* and *repleta* but nearer the size of the latter. Body build on the average a little toward the *repleta* type, squattier than *hydei hydei*. Type locality, Yucatan, Mexico. Cotypes in collection, College of Wooster.

On attempting a large number of crosses of *yucatanensis* with *hydei* the two forms were found to be fully inter-fertile. The salivary chromosomes of hybrid larvae were examined, and from many good preparations it was ascertained that there were no major inversions and no apparent loose pairing or other abnormality. If there are any differences in the salivaries of the two subspecies they are slight.

DROSOPHILA VIRILIS AMERICANA SPENCER

This subspecies has been described briefly by Spencer (1938) and a short account of its cytology has been given by Hughes (1939). The partial fertility of both male and female hybrids between *D. virilis americana* and *D. virilis virilis*, and the striking combination of morphological, physiological and cytological character differences between the two subspecies are of particular interest in the study of speciation in the genus. It is therefore our intention here to give a fuller account of collection records, subspecific characters and hybridization experiments than that which appeared in the brief abstract mentioned above. A detailed account of the cytology of the subspecies and hybrids is shortly to be published by Hughes, and we shall refer only briefly to facts given in the abstract cited above

(Hughes, 1939). An extended study of subspecies crosses and hybrid sterility is being made at present by Harrison Stalker at the University of Rochester.

Historical

In order to appreciate the significance of this case it will be necessary to review briefly the work which has been done on the genetics of *D. virilis*. As long as sixteen years ago Metz, Moses and Mason (1923) published a 94-page monograph on "Genetic Studies in *Drosophila virilis*." In their general introduction this observation is found:

> It is believed that the only method of obtaining reliable information on chromosome evolution is by means of genetic analysis combined with cytological observation. Ideal material for such a study would be provided by a group of species satisfying the following four requirements: In the first place, it should exhibit among its members a series of different chromosome groups; secondly, the species should be susceptible to intensive breeding under controlled conditions; in the third place, one or more of the species should be favorable for genetic analysis through the study of mutant races; and lastly the species should hybridize with another and give fertile hybrids.

There follows mention of various animal and plant groups in which hybridization occurs, but in which others of the above criteria are lacking. Then comes reference to mutation in *D. melanogaster* and extensive collections of species and tests of these made by Sturtevant and Metz for possible hybrids. To quote further: "The results of these tests were all negative, however. . . . It should be noted at this point that Dr. Sturtevant has recently succeeded in hybridizing *D. melanogaster* and *D. simulans* (Sturtevant, 1920) with very interesting results. For the purposes outlined above, however, the possibilities are limited. The two species are almost identical and appear to have identical chromosome groups, and in addition the F_1 hybrids are sterile, so that only mutant genes obtained in both species can be used for comparison." This monograph reports 41 mutant types in *D. virilis*.

To-day it is of interest to note these facts in connection with the above quotations. Except for *D. melanogaster*, there are more mutant characters known in *virilis* than in

any other species of this or any other genus; over 300 mutations have been reported. The genetic maps of the chromosomes of this species have more genes accurately placed than in any other form except *melanogaster*. To quote from the abstract cited above (Spencer, 1938):

Drosophila virilis americana differs from *Drosophila virilis virilis* in the following characters: more fusiform body, darker body color, larger eye, finer eye-pile, broader carina, heavier cloud on posterior cross-vein and reddish rather than gray pupa case. In the former pupation occurs in the culture medium rather than on the sides of the culture vial. *Americana* etherizes very rapidly in contrast to the other subspecies. . . . The cross matings give a few hybrid offspring. The mating, *virilis* female by *americana* male, is more often successful than the reciprocal. When *virilis* females are impregnated by *americana* males about 2 per cent. of the eggs develop. The hybrids, both males and females, are partially fertile when crossed *inter se* or back to either parent subspecies. The F_1 hybrid resembles *americana* more closely than *virilis*.

To the above may be added this from Hughes (1939):

Metaphase plates of larval ganglia cells also show striking differences. Both the male and the female of *D. virilis virilis* have five pairs of rod-shaped chromosomes and one pair of dots. In the *D. virilis americana* female there are two pairs of V-shaped chromosomes, one pair of rods and one pair of dots. The male of this subspecies has three V-shaped chromosomes, four rods and one pair of dots. Two of the rods in the male appear to form a pair corresponding to the one pair of rods in the female. The other two rods seem to pair with one of the V-shaped chromosomes, one with each arm.

Thus not only in the genus Drosophila but in the very species which formed the subject of this monograph are found for the first time the combination of the four requirements deemed necessary by its authors for the reliable study of chromosome evolution. To these may be added the new tool, salivary analysis. Nor does the fact that the hybridizing forms have been described as subspecies seem in any way to invalidate the case. On this very point Dr. H. J. Muller in a personal communication to the author writes:

To gain for your and your co-workers' studies on these two forms the respect and attention which I think is due, I therefore think that *americana* should be elevated to specific rank. Surely it does not, naturally, form an interbreeding population with *virilis*, whatever the reason for that may be, because it differs in so many and such marked characters at once, without intergrades having been found in nature. Hence even though when artifi-

cially bred a mixed population can be obtained, I think it should be called a different species, though one far better adapted by its crossability for the genetic study of species differences, than any hitherto obtained in *Drosophila*.

Collection Records

In the summer of 1936 the author set out a number of fermenting banana traps in open woodland and meadow along Sugar Creek about a mile from the village of Smithville, Wayne County, Ohio, and perhaps a quarter of a mile from the nearest farmhouse. These traps were visited once a week and flies taken to the laboratory and examined for species. On June 25 among the 97 flies taken there was one *virilis* female. On June 22, from a line of traps in the City Park, Wooster, Ohio, 125 flies were taken and among them one *virilis* male. These species collections are recorded in Table I. The *virilis* male was preserved in alcohol and on recent examination proves to be *virilis americana*. It becomes the first authentic specimen collected.

TABLE I

Species Collections of Drosophila Containing Virilis

	City Park, Wooster June 22, 1936		Sugar Creek Woods June 25, 1936	
Species	Males	Females	Males	Females
affinis	35	9	4	0
algonquin	15		6	
buskii	1	1	40	20
funebris	0	0	4	0
immigrans	1	1	1	0
melanica	1	0	0	0
melanogaster	1	3	3	9
putrida	1	0	0	1
robusta	26	22	7	1
transversa	1	6	0	0
virilis	1	0	0	1
Totals	83	42	65	32

From the female, which had been impregnated before capture, a stock was established. Some years before we had had stocks of *virilis virilis* in our laboratory and one of the most striking characters observed was the divergence of the anterior scutellar bristles which in most species then known to us are directed backward and parallel. It was by this character that the two *virilis* flies were first spotted in the collections. The *virilis* stock was assumed to be type

virilis and taken along with other Drosophila material to the California Institute of Technology in September. Shortly thereafter, Sturtevant and the author compared this stock with Japanese stock of *virilis* in that laboratory. It was at once obvious that the two were quite similar but differed in a number of definite characters. Experiments were then set up to determine the extent of these differences and the possibility of the two forms hybridizing.

In the summer of 1938, early in July, the author and Mr. Harrison Stalker made collections of Drosophila in and near the Great Smoky Mountains National Park. From traps exposed at Gatlinburg, Tennessee, near the west fork of the Little Pigeon river among over 2,000 flies taken there were 3 *virilis americana* females. In addition to these collection records, Sturtevant has in his pinned collection of Drosophila a specimen taken many years ago near Chattanooga, Tennessee, by W. S. Adkins, which, although badly damaged, agrees with *americana* in carina shape and clouding of the wing vein, and probably represents the first specimen of the subspecies to be captured. Among many thousands of Drosophila collected by Stalker and the author in the environs of Wooster no more specimens have been secured. It would appear that the species is either a rare one in this section or not drawn to the traps by the bait exposed. The first assumption seems the more likely, as laboratory observation has shown that this fly is attracted by and feeds and breeds on the yeasted banana bait used in the traps.

Concerning the geographical distribution of *virilis virilis* Kikkawa and Peng (1938) state: "The species is widely distributed and common in the eastern Palearctic regions, but very rare in Nearctic regions." They report collecting it at 40 different stations in Japan and Korea. Sturtevant (1921) mentions two collection records from the United States, one pair taken by himself in New York City in 1913 and a strain secured from a grocery store in Terre Haute, Indiana, by R. R. Hyde. In addition Metz, Moses and Mason (1923) mention a specimen taken in Los

Angeles. Because of the rarity of the American collections and the fact that they have all come from cities Sturtevant has suggested that the species is an introduced one. That *virilis virilis* may itself represent a complex of subtypes is indicated by certain observations. Kikkawa and Peng (1938) state: "Though no extensive study has yet been performed, the Japanese form is somewhat different from the American; namely, the former is longer than the latter in both the length of the body and of wing." They are here referring to American stocks of *virilis virilis* and not to *americana*. A Chinese stock received by us from Dr. M. Demerec crossed to *americana* with much more difficulty than did the Japanese stock used. When these were carefully compared it was found that the two *virilis virilis* stocks differed somewhat in carina shape, the Chinese being the broader.

It would appear, then, that *virilis virilis* is native to the Far East, China, Japan and Korea, and that *americana* is a subspecies which has a considerable range in the United States, though apparently a relatively rare form.

Morphological Characters

Virilis americana has eyes which are larger than those of *virilis virilis*. More striking than difference in eye size is the very short pile on the eyes of the former, giving them a smooth appearance. The width of the cheek is correspondingly greater in *virilis* than in *americana*. The carina of *americana* is broad and not at all sulcate, while that of all stocks of *virilis* examined is narrower and distinctly sulcate, although as mentioned above this character is variable. The abdomen of *americana* is more fusiform, giving the fly a "stream-lined" appearance. In making some hundreds of dissections of the internal organs of females of the two subspecies it has been noted that the viscera of *virilis* have a tougher texture, not so easily ruptured when put on a stretch. Four color characters have been noted. *Americana* has a darker body color, a slightly darker eye color and a lens-shaped dark cloud on the posterior cross-

vein in contrast to a narrow cloud of uniform width in *virilis*. The pupa case of *americana* is distinctly reddish in contrast to the dark grayish cast of the other form. Counts of the arista branches on both sides for males and females show that *americana* has a larger number of these branches. See Table II.

TABLE II

COUNTS OF ARISTA BRANCHES IN DROSOPHILA VIRILIS SUBSPECIES AND HYBRIDS. MEANS DERIVED FROM COUNTS OF BOTH ARISTAE OF 50 FLIES

	Parents	Males	Females
	americana male × *americana* female .	14.92 ± .18	15.64 ± .23
(a)	*virilis* male × *virilis* female	13.36 ± .20	13.28 ± .21
(b)	*virilis* male × *virilis* female	12.94 ± .12	13.40 ± .13
(c)	*virilis* male × *virilis* female	12.92 ± .12	12.90 ± .12
	virilis male × *americana* female	13.46 ± .14	13.18 ± .14
	americana male × *virilis* female	14.30 ± .12	13.86 ± .12
	virilis male × hybrid (v. male a. fem.)	13.22 ± .13	13.96 ± .19
	F_1 hybrid male × F_1 hybrid female ..	13.48 ± .18	14.12 ± .22

PHYSIOLOGICAL CHARACTERS

Five physiological character differences between the subspecies have been observed. *Americana,* after emergence from the pupa case, becomes sexually mature later than does *virilis*. Under the conditions of banana agar or cornmeal molasses agar culture *virilis* is more easily reared and more prolific than *americana*. With these culture media in glass containers *americana* pupates most frequently in the culture medium and *virilis* on the glass wall of the container and above the surface of the medium. See Table III. When several *virilis* flies of the same age are left undisturbed in a culture vial they tend to occupy isolated positions. *Americana* under like conditions tends to form more closely grouped aggregates. Finally, the

TABLE III

POSITION OF PUPA CASE IN SUBSPECIES AND HYBRIDS OF DROSOPHILA VIRILIS

Parents	Pupa touching or buried in food	Pupa on glass side of vial
virilis *americana* 3 vials	106	2
virilis *virilis* 3 vials	37	188
americana male × *virilis* female 6 vials, several pairs	40	2
virilis male × *americana* female 7 vials, several pairs	162	28

most striking physiological character noted is the etheri-

zation time. The author has at one time or another cultured an even 50 species and subspecies of Drosophila and of all these *virilis virilis* is without doubt the slowest to go under ether. At the other end of the series *americana* etherizes most rapidly, if we consider the time necessary to quiet the animal as etherization time. There are a few of these species which are killed by ether a little more quickly. Thus we have observed 17 morphological and physiological character differences, and it is quite possible that others might be found, particularly if internal organs were carefully studied. A thorough examination of the male external genitalia, however, has failed to show any differences here.

Subspecies Characters in the Hybrids

It might seem that since there is partial fertility in F_1, F_2 and backcrosses of hybrids of either sex to either parent subspecies this large number of character differences would offer excellent opportunity for a study of possible gene differences conditioning them and the localization of these genes by appropriate crosses using mutant markers. This seemed a particularly promising lead when it was found that in reciprocal crosses between the two subspecies the characters of *americana* proved to be dominant to those of *virilis,* except for carina width and arista count, which were clearly intermediate in the hybrids. Thus the hybrids could easily be distinguished from *virilis* but appeared almost identical to *americana.* However, it must be remembered that the majority of these characters are phenotypically less extreme than most mutants with which the Drosophilist works, or are of a physiological nature not easily workable. Further, a careful examination of several F_2 series indicated that most if not all of the characters amenable to studies on segregation were due to multiple factors. Thus an F_2 series showed gradations in carina width, eye size, eye pile, etc., which the author could not separate into segregating classes. We do not claim that such a study would be impossible. We do feel that at

the present stage of the Drosophila work it would be impracticable. If such a study were undertaken the first prerequisite would be to work out a set of culture conditions more nearly standard than those used in most laboratories. The work would require very careful quantitative measurements and statistical analysis. As the branches on the arista seemed a character lending itself to this type of study the author made complete arista branch counts on 897 flies, which totaled over 12,000 branches recorded, and included both subspecies, hybrids, F_2 hybrids, backcrosses of hybrids to both subspecies. Some of these data are shown in Table II. They indicate that there are more arista branches on *americana* and that the hybrids are intermediate. The subspecies do not differ sufficiently to make analysis of this type profitable except under very rigid environmental control. The (b) and (c) lots of *virilis virilis* were reared under more nearly optimum culture conditions than lot (a) or the *virilis americana* lot and along with F_1 hybrids show a lower standard error of the mean than do the F_2 hybrids.

Another character which is amenable to quantitative analysis is the position of the pupa case in relation to surface of the culture medium. Table III gives some data on this character in both subspecies and in the hybrids. However, such a character does not seem trustworthy for extensive analysis except under a very elaborate culture control. It is obvious from data presented in the table that the character is dominant in the F_1 hybrids, which are like *americana* and not intermediate to the two subspecies.

FERTILITY IN SUBSPECIES CROSSES AND IN HYBRIDS

When *virilis americana* and *virilis virilis* are crossed a very small per cent. of the eggs hatch, regardless of which direction the cross is made. It might be argued if the cross were made in mass culture that only a few of the females were impregnated. In order to clear up this point the following experiment was carried through. Eighteen *virilis* females were kept for several days with well-aged

americana males. These females were then isolated, placed in individual vials with no males present, egg counts made for two days, eggs given optimum culture conditions and females dissected at the end of this two-day period and the presence or absence of living sperm in the seminal receptacles recorded. The results are given in Table IV. The summary shows that the total number of eggs laid by flies giving any offspring was 336; total offspring from these flies 16; offspring from 4.7 per cent. of the eggs. The total

TABLE IV

FERTILIZATION OF EGGS IN CROSS OF AMERICANA MALES × VIRILIS FEMALES. SEE TEXT FOR DETAILS

Female No.	Number eggs laid	Adult offspring		Living sperm in females
		Male	Female	
1	39	0	1	Yes
2	45	0	0	Yes
3	83	1	2	Yes
4	74	2	6	Yes
5	accidentally killed			
6	89	1	1	Yes
7	57	0	0	No
8	85	0	0	Yes
9	71	0	0	No
10	109	0	0	No
11	91	0	0	Yes
12	35	0	0	No
13	69	0	0	No
14	70	0	0	Yes
15	51	0	2	Yes
16	102	0	0	Yes
17	74	0	0	Yes
18	78	0	0	Yes
Totals .	1,222	4	12	12 of 17

Total eggs from flies giving any offspring—336.
Total offspring—16. Offspring from 4.7 per cent. of eggs.
Total eggs from flies showing living sperm—881.
Total offspring—16. Offspring from 1.8 per cent. of eggs.

number of eggs from females showing living sperm at the end of the experiment was 881; total number of offspring 16; offspring from 1.8 per cent. of the eggs.

It may be definitely stated that there is no marked mortality in larval or pupal stages. Under proper environment eggs hatching go on through to the adult stage. The adults furthermore are quite normal in appearance. The cross here was made to the wild Japanese stock of *virilis virilis* females. While no such elaborate experiment has been carried through using the mutant stock ruffled-interrupted-peach of *virilis* as female parents it may be stated that on several occasions vials of *americana* to this mutant

strain have given much larger numbers of hybrids than when the wild *virilis* strain was used.

In the dissection of hundreds of females both in inter and intra subspecific crosses it has been noted that generally when a female contains any sperm in the intra cross her chitinous receptacles and ventral receptacles are packed full of sperm; while when a female in the inter cross contains sperm they are relatively few in number, the receptacles not being packed full. This seems to indicate either that copulation occurs less frequently between the subspecies or that it is shorter in duration and results in the emission of a small amount of sperm. Owing to the fact that the eggs which hatch go through to the adult stage, that the hybrids are very vigorous and normal in appearance, and that certain stocks cross more readily than others it seems likely that the partial sterility is due to psychological factors leading to partial sexual isolation rather than to any incompatibility of chromosome or genic complexes.

In order to test directly whether males mated with their own females in preference to those of the other subspecies the following experiment was done. Vials were made up containing 5 *americana* males with 5 *americana* females and 5 *virilis* females, all flies having emerged within the 24-hour period previous to the beginning of the experiment. If anything, this favored cross mating, as the *virilis* females mature more rapidly than the *americana females.* After the lapse of certain time intervals females were dissected and presence or absence of sperm recorded. The results are given in Table V. A similar but less extensive experiment using *virilis* males was carried through, and results are found in Table VI.

Reference to these tables will show the partial sexual isolation of the two subspecies when confined in small vials. From this and other experiments it seems that *virilis* males do not mate as readily with *americana* females as do *americana* males with *virilis* females. Another experiment may be cited to substantiate this point. In a half pint culture bottle containing culture medium there were confined 50

TABLE V

PREFERENTIAL MATING. AMERICANA MALES (5) × AMERICANA FEMALES (5) AND VIRILIS FEMALES (5) PER VIAL. FEMALES DISSECTED AT INTERVALS AND PRESENCE OF SPERM RECORDED

Vial No.	Time after start of experiment	*Americana* females		*Virilis* females	
		sperm	no sperm	sperm	no sperm
1	56 hours	0	5	0	5
2	106	0	5	0	5
3	135	0	5	0	5
4	156	4	1	2	3
5	156	5	0	3	2
6	156	4	1	1	4
7	156	2	2	2	2
8	156	5	0	2	3
9	156	5	0	5	0
10	156	4	0	1	2
11	156	3	0	1	3
12	156	4	0	5	0
13	180	4	1	5	0
14	180	0	5	0	5

Note: Vial 14 very moldy; shows that condition of food affects mating.

americana males with 50 females of each subspecies. At the end of 16 days surviving females were dissected and examined for presence of sperm. A similar experiment was

TABLE VI

PREFERENTIAL MATING. VIRILIS MALES (5) × VIRILIS FEMALES (5) AND AMERICANA FEMALES (5) PER VIAL. FEMALES DISSECTED AFTER 180 HOURS AND PRESENCE OF SPERM RECORDED

Vial No.	Time after start of experiment	*Americana* females		*Virilis* females	
		sperm	no sperm	sperm	no sperm
1	180 hours	2	3	5	0
2	180	0	4	5	0

carried out simultaneously, using *virilis* males. The results are given in Table VII.

TABLE VII

PREFERENTIAL MATING. 50 MALES WITH 50 FEMALES OF EACH SUBSPECIES KEPT TOGETHER IN HALF-PINT CULTURE BOTTLE FOR 16 DAYS. SURVIVING FEMALES DISSECTED AND PRESENCE OF SPERM RECORDED

Males used	Females *americana*		Females *virilis*	
	sperm	no sperm	sperm	no sperm
Americana	22	6	27	4
Virilis	12	22	38	0

One gets the impression from mass cultures of the hybrids mated *inter se* that they breed with greater ease than the original cross. However, when pair matings of hybrids of *americana* males to *virilis* females were made up, egg counts made and offspring emerging checked it was found

that some pairs showed complete sterility. Table VIII gives data secured from several pairs mated on January 31

TABLE VIII

FERTILITY OF PAIRS OF F_1 HYBRIDS FROM CROSS OF AMERICANA MALES TO VIRILIS FEMALES. MATINGS OF AGED HYBRIDS MADE ON JANUARY 31; EGGS COLLECTED FEBRUARY 3 TO 11

Pair No.	Total eggs	Total adults		Per cent. egg fertility
		Males	Females	
1	82	0	0	0.0
2	335	3	0	0.9
3	52	2	2	7.7
4	43	0	0	0.0
5	54	7	5	22.2
6	217	9	12	9.6
7	76	4	9	17.1
8	353	10	11	5.9

and from which eggs were collected over the period from February 3 to 11. These eggs were all normal in appearance and the low hatchability was probably due again to small amount of sperm, though no dissections were made. When F_2 hybrid pair matings were made the egg hatchability was even lower than with the original subspecies cross. Some of these females laid small, deformed and abortive eggs, indicating the probable segregation of abnormal factor combinations.

An experiment was run to test the fecundity (egg-laying capacity, of 12 hybrid females from cross of *virilis* female to *americana* male. Each female was placed in an egg-laying cage (Spencer, 1937) on January 31 and daily egg counts made till all females were dead. The results of this experiment show that at least some of the females have a high fecundity. The food used was banana agar, always fresh, and conditions were as nearly optimum as it was possible to supply, with temperature from 22 to 24 C. The egg counts were made daily, but in the table the data have been recorded in 11 egg-laying periods. Eggs from these females were normal in appearance, but they were unfertilized as no males were used in the experiment. The data are given in Table IX. It is not known why there was so much variability in the fecundity of these F_1 hybrid females. Nor do we have comparable

TABLE IX

FECUNDITY OF 12 F_1 HYBRID FEMALES FROM CROSS OF AMERICANA MALE × VIRILIS FEMALE. DAILY EGG COUNTS MADE BUT RECORDED IN TABLE IN 11 PERIODS. J REFERS TO JANUARY; F—FEBRUARY; M—MARCH

Hybrid female No.	J31 to F4	F5 to F12	F13 to F18	F19 to F25	F26 to M3	M4 to M9	M10 to M16	M17 to M28	M29 to A3	A4 to A8	A9 to A16	Totals eggs	Died
1	93	152	338	264	84	79	91	39	0	1	..	1,141	April 7
2	39	54	2	11	0	17	0	0	...	...	..	123	March 13
3	296	488	335	349	344	384	447	290	290	205	50	3,478	April 16
4	51	88	61	133	6	...	...	...	...	...	..	339	March 3
5	0	82	219	121	224	112	122	118	35	35	7	1,075	April 15
6	0	193	132	0	0	1	0	0	0	0	0	326	April 20
7	143	265	285	167	...	...	...	...	...	...	..	860	February 25 (accidental)
8	93	94	30	78	13	19	14	0	0	...	..	341	March 30
9	17	16	70	276	188	18	2	7	0	...	..	594	March 30
10	269	565	396	158	90	40	44	17	0	...	..	1,579	April 3
11	34	32	7	15	3	...	...	...	...	...	..	91	February 28
12	44	43	0	0	0	...	...	...	...	...	..	87	February 27
Totals	**1,079**	**2,072**	**1,875**	**1,572**	**952**	**670**	**720**	**471**	**325**	**241**	**57**	**10,034**	

data on females from the two subspecies. These data are presented to show that some hybrids have high fecundity, and also to place on record an experiment on egg-laying capacity in Drosophila under optimum food conditions. The total of 3,478 eggs laid by hybrid female No. 3 is far in excess of the highest egg-laying record previously reported in Drosophila.

GENETIC CROSSING-OVER IN HYBRID FEMALES

Early observations on the salivaries of the hybrids indicated that there were major inversions in some of the chromosomes as well as much loose pairing. Such a condition would be expected to disturb cross-over ratios. In order to test this point we wrote to Dr. M. Demerec for the best multiple mutant stocks then available at the Cold Spring Harbor laboratory. Unfortunately only suitable material for testing the first and fifth of the five long chromosomes was available. When crosses of these and several single mutant stocks to *americana* were made it was found that there was a great deal of difference in the ease with which hybrids could be secured from the several crosses. The fifth chromosome stock, ruffled-interrupted-peach crossed to *americana* more easily than did any of the other mutant stocks used. In contrast some others were very difficult to cross, and in fact from some no hybrids were secured. It is quite apparent that there are factors present in the mutant stocks of *virilis virilis* which have to do with inter-subspecific fertility. Our data on crossing-over together with some secured by Mr. Robert Baxter in our laboratory indicate that cross-over values are greatly modified in those parts of chromosomes one and five which have been tested. Suitable stocks for testing of cross-over values for most of the *virilis* chromatin have not been available to us.

SUBSPECIES AND SPECIATION IN DROSOPHILA

The author's designation of the new *virilis* form as a subspecies has been questioned both on the grounds that

it should be given specific rank and also by those who would place it in a taxonomic rank below that of subspecies. For this and other reasons it seems pertinent to review briefly those cases in the genus Drosophila where hybrid formation has been found possible.

Evidence is rapidly accumulating to indicate that gene and chromosomal mutations are present in abundance in wild Drosophila. Thus at the primary level of inherited variation Drosophila populations may be thought of as fluctuating combinations of gene frequencies and chromosomal sequences. It may be asked whether point mutations or diverse chromosomal sequences ever become sufficiently established to constitute local races. The work of Dobzhansky and Sturtevant (1938) proves that populations of pseudoobscura with varying percentages of chromosomal sequences do occur. It seems by inference probable that over the range of this and other species local populations pure for certain chromosomal sequences exist and differ in this respect from other local populations. Gene mutations have also been known to reach a high percentage frequency in local populations. For example, Sturtevant (1915) has reported a sex-linked gene, light body color, in *D. repleta,* wide-spread and making up an appreciable part of certain collections of this species. Last summer this species was recorded for the first time from Wooster, Ohio. Mr. Stalker brought in a trap which had been exposed for some time and which contained adults and larvae of *repleta* with roughly two thirds of the gene light to one third so-called wild-type. As the species is rare in this region it is quite conceivable that a small local population could be carried through the winter by a few light individuals and become homozygous light. Spencer (1938) has reported high frequencies of bobbed genes in various *hydei* populations. But these several gene and chromosomal changes appear to be perfectly interfertile with the so-called wild-type and are thus subject to contamination even after pure races are established.

There is apparently little difference between the estab-

lishment of a local race differing from the type of the species by one gene and the isolation and fixation of a local race differing in an ensemble of characters conditioned by several mutational changes. This, in fact, appears to be the evolutionary status of *D. hydei yucatanensis,* a form perfectly interfertile with *hydei hydei,* but differing from the latter by several inherited characters not associated with hybrid sterility or gross chromosomal rearrangements. In a similar category may be placed the subspecies of *affinis* and *athabasca* described by Sturtevant and Dobzhansky (1936). In both of these cases the descriptions of subspecies are based upon slight inherited color differences found in different parts of the geographical range of the species.

Let us now consider cases in which there is a range from partial to complete hybrid sterility in intercrossing forms. As described above the cross *virilis virilis* × *virilis americana* gives hybrids which are partially fertile in both sexes though the initial cross is made with difficulty. The principal isolating mechanism when crosses are attempted appears to be psychological. In nature geographical isolation is also a factor. The hybrids of both sexes are partially fertile. Next in order as regards hybrid sterility is the cross between the morphologically identical Race A and Race B of *pseudoobscura* (Lancefield, 1929). The hybrid females are partially fertile when crossed back to males of either race; the hybrid males are completely sterile. Finally there are the crosses between melanogaster and simulans (Sturtevant, 1919), *pseudoobscura* and *miranda* (Dobzhansky, 1935), and *azteca* and *athabasca* (Sturtevant and Dobzhansky, 1936) which give completely sterile hybrids. Further in these cases there are disturbances in the sex-ratio of the hybrids and certain morphological abnormalities in one or both sexes. Thus we have in nature in this genus a series of cases ranging from variants at the single gene mutation and chromosome inversion level, through completely interfertile forms differing by several morphological characters, to

restricted interbreeding types with partial fertility of hybrids of both sexes, complete sterility of hybrids of one sex, and finally complete sterility of hybrids of both sexes. In addition the genus contains groups of species which although intersterile are so nearly alike morphologically that they can only be distinguished by careful attention to minute morphological characters.

Any attempt to classify these forms in an ascending hierarchy of genetic relationships is bound to meet with difficulties. To suppose that the cases mentioned here can be fitted into any static scheme involving fixed levels of variety, race, subspecies and species is to lose sight of the dynamic nature of the process which brought them about. To simplify we may consider only those cases where two forms cross with some difficulty and give hybrids which show some degree of sterility. Conceivably these may be seriated in regard to: differences in morphology and physiology; in psychology and sex reactions; in salivary chromosome structure; in gonial chromosome groups; in hybrid fertility; and in geographical and ecological distribution. An attempt at this has been made in Table X. Obviously this is only an approximation. For one thing the variation of strains within the race or subspecies in reactions to another race makes it impossible to seriate accurately even for one category. Furthermore, the author's information has been gleaned partly from published descriptions and no really accurate comparison can be made without first-hand manipulation of the living material. It seems clear, however, that within this genus evolution has progressed and is progressing along diverse lines, rapidly in one respect and more slowly in another.

In conclusion, we may question the validity of calling the two forms of *pseudoobscura* races, *melanogaster* and *simulans* or *miranda* and *pseudoobscura* species, or *americana* and *virilis, hydei* and *yucatanensis, athabasca* and *mahican* subspecies. After all, what does it matter? It might be well for Drosophilists to formulate some work-

TABLE X

SERIATION OF CASES OF HYBRIDIZING FORMS IN DROSOPHILA. MOST SIMILAR TYPES AT THE LEFT GRADING TO MOST DIVERSE TYPES AT THE RIGHT

Differences based on					
Morphology and physiology	*Pseudoobscura* A *Pseudoobscura* B	*Miranda* *Pseudoobscura*	*Athabasca* *Azteca*	*Melanogaster* *Simulans*	*Americana* *Virilis*
Salivary chromosomes	*Melanogaster* *Simulans*	*Pseudoobscura* A *Pseudoobscura* B	*Americana* *Virilis*	*Miranda* *Pseudoobscura*	*Athabasca* *Azteca*
Gonial chromosomes	*Athabasca* *Azteca*	*Pseudoobscura* A *Pseudoobscura* B	*Melanogaster* *Simulans*	*Miranda* *Pseudoobscura*	*Americana* *Virilis*
Sex reactions	*Pseudoobscura* A *Pseudoobscura* B	*Miranda* *Pseudoobscura*	*Athabasca* *Azteca*	*Americana* *Virilis*	*Melanogaster* *Simulans*
Hybrid sterility	*Americana* *Virilis*	*Pseudoobscura* A *Pseudoobscura* B	*Miranda* *Pseudoobscura*	*Melanogaster* *Simulans*	*Athabasca* *Azteca*
Geographical distribution	*Melanogaster* *Simulans*	*Miranda* *Pseudoobscura*	*Pseudoobscura* A *Pseudoobscura* B	*Athabasca* *Azteca*	*Americana* *Virilis*

ing principle for classifying, but perhaps the ink would hardly be dry when a new case outside the rules would be discovered. Apparently we are at last in a position to see species, subspecies, racial and varietal lines breaking down before the advances of field and laboratory study of evolution in the making.

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SHORTER ARTICLES AND DISCUSSION

CRASPEDACUSTA AGAIN—

EARLY in September of the present year several persons called the writer's attention to the occurrence of jellyfish in the water of Cheat Lake. The lake was at once visited, and half a dozen living specimens were obtained, after some effort, with the aid of a student, James Hunter, and his rowboat.

It was reported by other observers at the time that "thousands" of the medusae had been seen in the same vicinity. Another collection of seven living specimens was brought to the laboratory by another student a few days later.

My identification of the medusa as *Craspedacusta sowerbii*, the only fresh-water species found in the United States, was verified by Dr. Waldo L. Schmidt, of the United States National Museum.

Cheat Lake is an artificial body, formed by the damming, about thirteen years ago, of the Cheat River by a power company. It is about seven miles long and has an average width of about one fourth mile. The surface of the water is 860 feet above sea level. The specimens in question were found near the Ice's Ferry Bridge, seven miles northeast of Morgantown.

This is the fourth report of *Craspedacusta* from West Virginia: Professor P. C. Bibbee reports it from Wood County, on the western border of the state and from Mercer County on the southern border; Professor P. J. Zucchero reports it from Fayette County in the center of the state; and the present find is in Monongalia County on the northern boundary of the state.

According to Schmidt,[1] the medusa has been found in twenty states of the Union, in the District of Columbia and in the Canal Zone.

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STUDIES ON THE CHEMOTHERAPY OF CANCER IN MICE DERIVED FROM GENETIC PRINCIPLES

IT is the purpose of this paper to outline, in brief, the development of cancer research as conceived some twenty years ago and executed uninterruptedly up to the present time. The original

[1] AM. NAT., January–February, 1939.

investigation of the production of uniform stocks of experimental mice was undertaken for two reasons: (1) to eliminate or to standardize biological variability that unquestionably influences the onset and fate of malignancy within the organism and (2) to provide a supply of animals that give rise to spontaneous tumors. It may be recalled that this work was started when spontaneous tumors in mice were very rare and even before the induction of tumors by pure chemicals had been placed on a mass production basis. There is still a prevalent feeling among cancer investigators that the study of the origin of spontaneous tumors in experimental animals may lead to clearer concepts of the fundamental nature of the disease than some of the present investigations on the induction of tumors.

The use of standardized stocks of mice made possible the analysis of susceptibility and resistance to transplantable tumors. It was possible to demonstrate that these phenomena were strictly inherited according to the fundamental genetic laws as postulated by Mendel. Two concepts on the nature of cancer were formulated from this work, as follows: (1) the cancer cell is controlled by an intrinsic or genetic constitution which underlies its physiological behavior and (2) the genetic constitution of the tumor cell has deviated, by a process analogous to somatic mutation, from the genetic constitution of the tissue of the body from which it has originated. That is, it is reasonably clear that the origin of cancer, brought about by a diversity of physical and chemical agents, such as x-rays, severe burns, benzpyrene, o-aminoazotoluol, etc., or by unknown agents that lead toward spontaneous cancer, is, in the last analysis, brought about by a response or reorganization of the genetic constitutional factors within the cell which control the physiological functions of that cell. If this concept be valid, based upon indirect rather than direct experimental evidence, then certain agents known to have an effect on genetic phenomena within cells ought to or may have an effect on the origin and fate of neoplastic cells. Among those agents which have been demonstrated to have an effect upon genetic phenomena within cells is temperature. It has been shown that temperature, under which Drosophila (the common fruit-fly) are kept, has a pronounced effect upon crossing-over, that genetic phenomenon within cells upon which the concept of the linear order of the genes and the architecture of the germ plasm is based. The experimental regulation of different temperatures to localized

parts of an organism in which the heat mechanism is very carefully controlled, such as it is in mammals, is more difficult than it is in a cold-blooded animal such as Drosophila. Consequently, the best one can do is to analyze or to standardize some of the component agents which bring about the regulation of temperature. Among these agents is hemoglobin, the oxygen carrier to the tissues of the body. The amount of circulating hemoglobin is, at any one time, a resultant of the pouring into the blood of new supplies of the material and the using or dissipation of the material during its physiological functioning.

In collaboration with Dr. T. H. Werner, an investigation was set up in order to determine shifts of hemoglobin per unit of time in those individuals known by past experience to differ in their intrinsic ability to give rise to, or not to give rise to, spontaneous tumors of the mammary gland. It was clearly shown that there was a close correlation between hemoglobin readings per unit of time and this intrinsic genetic propensity to develop spontaneous tumors. Eight different genetic strains of mice were used; 3 of these developed tumors of the breast very early in life (A, C_3H and D); 2 strains developed tumors at advanced ages—22 to 26 months of life (C and CHI); and 3 were, under the conditions of the laboratory, relatively resistant to the same disease of cancer (N, JK and CBA). The 3 susceptible-to-carcinoma strains gave fundamentally the same type of curve (a gradually decreasing type beginning at 150 days of life); the 3 relatively resistant strains gave a uniformly different curve per unit of time (a rapidly increasing type up to the time when spontaneous tumors were occurring in the susceptible strains, 350 to 450 days of life); while the 2 strains of an intermediate degree of susceptibility to the onset of malignancy gave an intermediate type of hemoglobin shift per unit of time between the highly susceptible and the relatively resistant strains. It was concluded that this fundamental shift in the hemoglobin level of the blood may be an index of the physiological aging process within the organism and may be part of the genetic constitution of the mouse (quite distinct from the genetic constitution of localized cells of the body) in relation to the onset of malignancy that may be determined by genetic factors.

The lowest curve of the 3 resistant-to-cancer strains was obtained with the CBA mice. The highest curve of the 3 susceptible-to-cancer strains was found in the A mice. Consequently, if this

difference could be proven to be statistically significant, then the trends in hemoglobin with the other classes where a greater variation in readings is present, is, more than likely, significant. When opportunity arose to repeat the hemoglobin work under entirely different laboratory conditions, the CBA and A strains were selected. This work was done in collaboration with Dr. L. D. Francis. The conclusion has recently been verified from this work done at Yale, that there is a precocious drop in hemoglobin in those mice which are susceptible to give rise to spontaneous tumors of the mammary gland.

Another conclusion of the orginal work, done in collaboration with Dr. T. H. Werner at Bar Harbor, was that there is not only a fundamental difference in the shift of hemoglobin level per unit of time between those mice which are genetically susceptible to give rise to tumors and those which are genetically resistant, but also another difference in the shift of hemoglobin correlated with the number of hours the mouse has been separated from food. That is, a young individual of any strain of mouse has an ascending type of curve for hemoglobin shift between 2 and 6 hours following separation from food. With advancing age, this curve shifts to a descending type. This shift is probably part of that of the diurnal rhythm of hemoglobin which is of frequent occurrence in mankind, the significance of which is quite unknown. Now, it was determined that the change from the ascending to the descending type of curve took place at quite different chronological ages in mice of the different strains. This change is somehow or other correlated with a susceptibility to give rise to spontaneous tumors, since it occurs earlier in life in mice of those strains which were susceptible to spontaneous tumors. It may also be a reflection of the physiological aging process which is unquestionably a powerful force in the origin of spontaneous tumors.

Since a standard oatmeal mixture was the diet upon which all experimental mice were kept at that time, an attempt was made to investigate the influence of certain chemicals added to this otherwise basic diet. It was found that by the subtraction of salt from the basic diet, an elevation of hemoglobin within a few hours was obtained. Similarly, the addition of certain of the essential oils, particularly oil of lemon (citral), oil of gaultheria and oil of vanillin, produced an elevation of hemoglobin within a few hours on human subjects.

The nature of this hemoglobin shift has not as yet been deter-

mined. One or more of several conceptions may be valid, as follows: (1) the shifts of hemoglobin may be due to dehydration phenomena within the body, (2) the availability of food materials to the tissues of the body may be diminished (it is known that oil of gaultheria and other of the essential oils slow up enzymic activity, particularly in the alimentary canal), or (3) the oxidation-reduction potentials of the tissues may be impaired or altered (the essential oils, particularly the ones used, contained, in addition to other chemicals, the aldehydes, which are powerful reducing agents). Perhaps some other explanation of the phenomenon observed is the true one.

The next step was to add small amounts of the common essential oils to the diet of those mice which were susceptible to the origin of malignancy. When this was done, it was discovered that the onset of cancer could be delayed, and in some cases actually prevented, by adding either (1) citral or (2) oil of gaultheria or (3) oil of thyme or (4) oil of allspice to the otherwise normal control diet. Several other of the essential oils have also been tested with varying degrees of success. Since most of the naturally occurring essential oils are mixtures of several components, it was necessary to restrict the development of the research to one. For this reason, the oil of gaultheria, containing, as it does, only 5 identified components, was selected.

Malignancy can be influenced by adding small amounts of the natural oil of gaultheria to the diet of a mouse after the spontaneous tumor has originated. No such effect could be obtained when the synthetic redistilled methyl salicylate was used. Further work disclosed the fact that the active agent in the natural oil of gaultheria which brought about liquefaction of spontaneous tumors was contained in the low boiling point fraction of that oil. The other two boiling point fractions obtained by fractional distillation of the true oil were, like the synthetic methyl salicylate, quite ineffectual in bringing about similar changes in tumors. Since heptyl aldehyde is contained in the low boiling point fraction of the true oil of gaultheria, other mice with spontaneous tumors were placed on diets containing this agent. Pronounced liquefaction changes and complete regression of some tumors occurred under this treatment. It was found that the heptyl aldehyde alone was not as effective as the low boiling point fraction in bringing about retrogressive changes in spontaneous tumors. Consequently, another series of mice were put on a diet con-

taining 3 parts of heptyl aldehylde to one part of methyl salicylate. This combination is the most effective way so far discovered in bringing about retrogressive changes in spontaneous tumors. Twelve of 45 mice used actually completely regressed their spontaneous tumors under this experimental treatment.

Twenty dogs with various types of spontaneous tumors have so far been treated with heptyl aldehyde. Complete regression of the original tumors have already occurred in several animals.

The last experiment that will be presented at this time consists of the periodic inoculation of heptyl aldehyde into mice that are known to be susceptible to the development of spontaneous carcinomas of the lungs. Of 60 mice, that have died so far not a single one has had a spontaneous tumor. The controls, on the other hand, continue to develop lung carcinoma at the expected rate. It seems clear, therefore, that the introduction of heptyl aldehyde into a susceptible mouse, either by diet or by injection, influences the mechanism involved in the origin of cancer.

SUMMARY

The present paper consists of the logical development of a conception of the origin of cancer in experimental animals over a period of 20 years. Not all the steps involved in the development of the theory have been completely demonstrated. Concrete results in the treatment and control of cancer in both mice and dogs are now being obtained. The research has reached the point whereby the following conclusions are valid: (1) malignancy in experimental animals has been significantly influenced, (2) the control of cancer in mice, at least, may eventually be had by chemo-therapy and (3) such a control of the disease under controlled conditions must in the last analysis solve, or throw considerable light upon, the fundamental nature of cancer itself.

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A NOTE ON ABNORMAL SHAPE OF EGG[1]

FULLY formed and complete eggs that deviate radically from the normal shape range are frequently encountered in farm flocks. It has been observed that hens, which have presumably entered the trapnests to lay but are prevented from doing so by

[1] Contribution No. 126, Department of Poultry Husbandry.

removing them to classrooms or into other strange quarters, frequently lay eggs which are odd in shape if time of laying is substantially delayed. These abnormalities are confined to the second egg, the first having the shape characteristic of the eggs for that hen.

That the premature removal of an egg from the duct does not influence the time of the succeeding ovulation has been demonstrated by Warren and Scott (1935). Although experiments

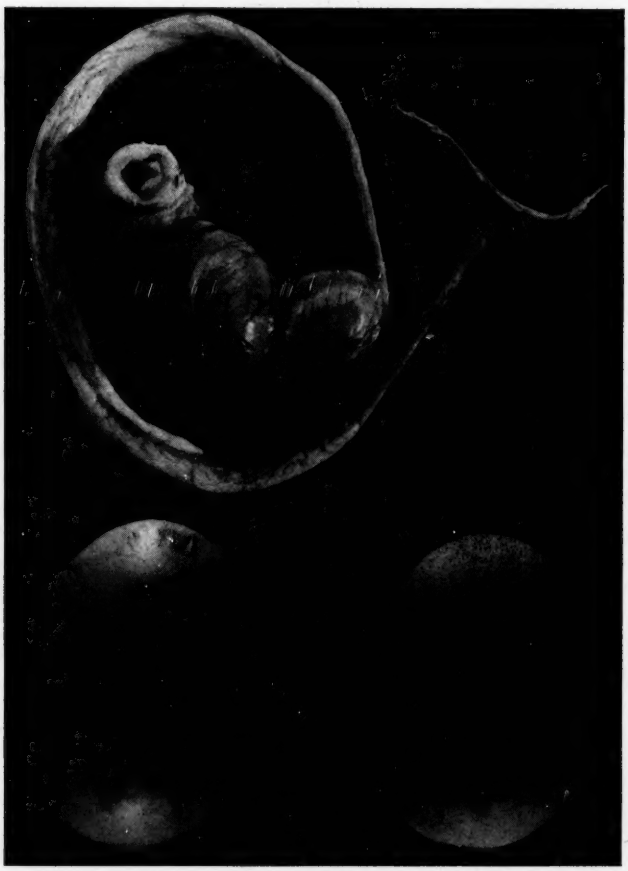

FIG. 1. Abnormal shape of egg resulting from the presence of two eggs in the fowl's shell-gland.

have not been conducted to show that a delay in oviposition likewise exerts no influence on time of ovulation we believe that such is the case, since the presence of two eggs in the oviduct has been detected repeatedly under these circumstances by palpation and confirmed at necropsy.

Usually the "held-egg" and the succeeding egg are laid on the same day and this has been interpreted by some workers as a heightened state of ovarian activity following injections of gonadotropic preparations. Not infrequently the second egg is prematurely expelled while still in a membranous state and practically devoid of calcium deposits. Although lacking a calcareous shell the egg is distinctly abnormal in shape. The area of the membrane adjacent to the shell of the "held-egg" is usually flattened. Moreover, the membranous egg retains its abnormal shape, even though the hydrostatic pressure is greatly increased by immersing in Ringer's solution. It is therefore evident that the elasticity of the shell membranes is modified shortly after they are secreted and that the abnormality resulting from the contact of the two surfaces can not be corrected by the imbibition of uterine secretion.

In view of these observations it would seem that modifications in egg shape resulting from operations upon the isthmus and uterus as reported by Asmundson and Jervis (1933) could be explained on the basis that a constriction was formed at the site of the operation which would have the same effect as two eggs in the oviduct. This would be particularly true for the isthmus whose lumen is small.

The available evidence would indicate that when carried to term chalazae formation is normal in the second egg. Their points of attachment and size are similar in every respect to those of the "held-egg." The amount of calcareous shell is, however, distinctly less than that found in the "held-egg," as shown in Table 1. Although the absolute weight of the shell of the "held-egg" was greater than that of the normal egg the relative amounts are almost identical, and therefore the former does not

TABLE 1

DATA CONCERNING THE AMOUNT OF SHELL IN THREE TYPES OF EGGS

	Normal egg			Held egg			Abnormal egg		
Hen	5398	5326	5392	5398	5326	5392	5398	5326	5392
Egg weight	55.6	67.9	...	60.0	67.6	62.1	59.1	55.4	61.4
Shell weight ...	6.1	6.5	...	6.4	7.2	6.5	4.4	4.9	5.1
Per cent. shell ..	11.0	9.6	...	10.7	10.7	10.5	7.4	8.8	8.3

appear to have acquired more than its normal complement of shell.

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STENOTHERMY AND EURYTHERMY OF ANIMALS IN RELATION TO HABITAT

IT is in the relatively narrow range of temperatures between the freezing point of natural waters and 40° C. that most poikilothermal animals carry on their vital activities. In any one species this range may be considerably less, and vary according to conditions in the environment. If a species can tolerate a wide range of temperatures it is said to be eurythermal. If the difference between the maximum and minimum limiting temperatures is small, this condition is referred to as stenothermy. Ecologists have appreciated in a general way that stenothermal animals are characteristic of environments where temperature fluctuations are small and eurythermal animals of environments where temperature fluctuations are great, but it is only in recent years that satisfactory experimental work has begun to appear on this subject.

It is the purpose of this review to compare the range of temperatures over which normal development is possible in aquatic and terrestrial animals. In aquatic environments temperature fluctuations are small compared with those occurring on land in the temperate zone. An attempt will be made to put on a more factual basis the hypothesis that stenothermal animals exist chiefly in environments with small temperature variations, and that eurythermal animals are found under more variable temperature conditions. The data to be presented are chiefly from the literature. Many references have been omitted due either to lack of rigid temperature control or if experiments were not begun early in development. This latter precaution is necessary as resistance to heat and cold frequently increases with the stage of development. However, all cases have been included where temperature control was adequate and where experiments were

begun either at fertilization or very early in development. The normal temperatures are those permitting at least 50 per cent. survival or, in certain cases where mortality is large at all temperatures, 50 per cent. of the maximum survival.

In Table 1 the minimum and maximum temperatures, together with the range (number of degrees over which normal develop-

TABLE 1

THE RANGE OF TEMPERATURE OVER WHICH DEVELOPMENT IS NORMAL IN AQUATIC ANIMALS

Species	Authority	Minimum °C.	Maximum °C.	Range °C.
Crustacea:				
Lernaea elegans	Nakai (1)	20.4	29.8	11
Moina macrocopa	Terao and Tanaka (2)	8.7	37.7	30
Mollusca:				
Dendronotus frondosus	Runnström (3)	− 1	11	13
Ostrea gigas	Senō, Hori and Kusakabe (4)	18.6	27.7	10
Mytilus edulis	Runnström (3)	4	16	13
Mytilus edulis f. galloprovincialis	Runnström (5)	8	23	16
Echinodermata:				
Cucumaria frondosa	Runnström (3)	− 1	11	13
Asterias glacialis	Runnström (5)	8	23	16
Asterias rubens	Runnström (3)	5	16	12
Paracentrotus lividus	Hörstadius (6)			
Winter		8	23	16
Summer		16	29	14
Paracentrotus lividus	Runnström (5)	8	29	22
Strongylocentrotus dröbachiensis.	Runnström (3)	− 1	11	13
Arbacia aequituberculata				
Early summer	Runnström (7)	14	29	16
Winter	Runnström (5)	8	29	22
Spatangus purpureus	Runnström (5)	8	23	16
Echinus esculentus	Runnström (3)	4	16	13
Echinus acutus	Runnström (5)	8	22	15
Echinocardium flavescens	Runnström (3)	7	22	16
Echinocardium cordatum	Runnström (3)	9	23	15
Echinocyamus pusillus	Runnström (3)	8	23	16
Psammechinus miliaris	Runnström (3)	8	22	15
Psammechinus microtuberculatus.	Runnström (5)	9	23	15
Sphaerechinus granularis	Runnström (7)	9	29	21
Tunicata:				
Ciona intestinalis	Runnström (3)	8	23	16
Ascidia mentula	Runnström (7)	8	22	15
Phallusia mammillata	Runnström (5)	8	23	16
Ascidiella aspersa	Runnström (5)	8	22	15
Ascidiella scabra	Runnström (5)	8	22	15
Corella parallelograma	Runnström (5)	8	22	15
Pisces:				
Pleuronectes platessa	Johansen and Krogh (8)	0	14	15
Pleuronectes platessa	Runnström (3)	3	14	12
Scomber scombrus	Worley (9)	11	21	11
Gasterosteus aculeatus	Leiner (10)	8	23	16
Pagrosomus major	Kajiyama (11)	13.9	20.8	8
Leuciscus hakuensis	Kawajiri (12)	9.2	19	11
Leuciscus hakuensis	Nakai (13)	9.7	17.2	9
Salmo irideus	Kawajiri (14)	3.1	13.1	11
Hypomessus olidus	Higurashi and Nakai (15); Nakai (16)	6	17.5	13
Calotomus japonicus	Senō, Ebina and Okada (17)	19.9	27.6	9
Oncorhynchus masou	Kawajiri (18)	5.8	13.0	8
Plecoglossus altivelis	Nakai (19)	9.3	22	14
Gadus callarias	Johansen and Krogh (8)	− 1	10	12

ment is possible), are given for a number of aquatic animals. This range is 16° or less in 91 per cent. of the cases. The distribution is as follows: 12 per cent. have a range of 8°, 9° or 10°; 30 per cent. have a range of 11°, 12° or 13°; 49 per cent. have a range of 14°, 15° or 16°; and 9 per cent. have a range of 17° or more.

The form with the greatest range, *Moina macrocopa*, although aquatic, should probably be included with the species undergoing wide temperature fluctuations, as it inhabits very small bodies of water, the temperature of which follows atmospheric conditions closely. With this form excluded, the average of the ranges for aquatic forms listed in Table 1 is found to be 14° C.

In spite of the vast amount of published data on embryonic temperature tolerance of terrestrial animals but little has been included in Table 2. Insects, which have received the greatest

TABLE 2

THE RANGE OF TEMPERATURES OVER WHICH DEVELOPMENT IS NORMAL IN TERRESTRIAL AND SEMI-AQUATIC ANIMALS

Species	Authority	Minimum °C.	Maximum °C.	Range °C.
Arthropoda:				
Cimex lectularius	Geisthardt (20)	13	36	24
Cimex rotundatus	Geisthardt (20)	16	37	22
Amphibia:				
Rana fusca	Hertwig (21)	1	24	24
Rana sylvatica	Moore (22)	2.5	24	23
Rana pipiens	Moore (22)	6	28	23
Rana palustris	Moore (22)	7	30	24
Rana clamitans	Moore (22)	11	35	25
Rana sphenocephala	Moore (23)	12	34	23

attention, are not especially suitable for studies of this kind. Early stages in development are frequently difficult to tell in the living egg, and consequently studies usually begin on eggs of unknown ages. Moreover, many insect eggs remain dormant at low temperatures and do not begin development until some critical temperature is passed. There is a distinct need for accurate data on the effect of temperature on development. Too often in the past investigators have been more interested in a particular theory of temperature action than in collecting accurate data to test such a hypothesis.

In Table 2 the maximum and minimum temperatures for two arthropods and six amphibians are given. It will be seen at once that there is great uniformity in the range over which normal development is possible, it being 22–25° C. in all cases and aver-

aging 23.6° C. This average is 68 per cent. greater than for purely aquatic animals. In view of the small number of cases given in Table 2 it might be well to point out that the work of Kobayashi (24) on *Musca*, Krogh (25) on *Tenebrio*, Ludwig (26) on *Popillia*, Ludwig and Cable (27) on *Drosophila*, Peairs (28) on many insects, Sanderson and Peairs (29) on many insects, Shelford (30) on the codling moth, Wadley (31) on *Toxoptera* and Wardle (32) on *Lucilia* all point to an embryonic temperature range of 20° or more for the forms studied. They have been excluded from the table because the temperature limits are not accurately determined. To these might be added additional data on amphibians (23). *Ambystoma maculatum* will develop at 3.5° and 23°; *Bufo americanus* at 10° and 30°; *Hyla crucifer* at 6° and 28°. The exact maxima and minima have not been determined in these cases, but a range of 20° or more is indicated in all. A range of this magnitude also exists in *Triton alpestris* (33), *Bufo bufo* (34) and *Pelobates fuscus* (34).

If stenothermal animals are arbitrarily defined as those having an embryonic temperature tolerance range of less than 19° C., and eurythermal animals as those having a range of 19° C. or more, we find that in 91 per cent. of the cases aquatic animals are stenothermal, and all the terrestrial and semi-aquatic species listed are eurythermal. The former live in environments where temperature fluctuations are small, and the latter where these fluctuations are large.

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